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THE PODOSTEMACEAE OF THE NEW WORLD II

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INTRODUCTION

In my first paper (1951) a part of the tribe Eupodostemeae was revised, viz. the genera Apinagia, Marathrum, Rhyncholacis, Lophogyne, Monostylis, Jenmaniella, Wettsteiniola and Macarenia.

The second part deals with the subfamily Tristichoideae, which comprises the genera Tristicha and Weddellina, and the tribe Mourereae of the subfamily Podostemoideae, which consists of the genera Mourera, Lonchostephus, and Tulasneantha.

In this part a revision of these genera is given; a list of the collectors' numbers mentioned in the second part and a general index are included.

The third part will deal with a part of the tribe Eupodostemeae, viz. the genera Oserya, Devillea, Ceratolacis, Mniopsis, Podostemum and Castelnavia. It will also give an account of the dubious genera, and it will contain additions and corrections to part 1, latin descriptions of new taxa in part 3, a list of the collectors' numbers mentioned in that part, new references to the literature, plates with explanatory text and a general index to the third part.

The herbarium abbreviations are those proposed in the "Index Herbariorum" of Lanjouw and Stafleu (1952).

The references to the literature are given in an abbreviated form consisting of the author's name followed by the year in which the publication appeared e.g. "Pulle (1906)" means "A. Pulle: Enum. vasc. pl. Suriname, (1906)". The place of publication can be found

in the list of references which is included in the first part. Those not mentioned there are found in the third part.

The following abbreviations are used:

„Fl" the time of the year at which flowering specimens were collected.
„Fr" the time of the year at which fruiting specimens were collected.

When the name of a month is given without one of the prefixes fl or fr, it means that the specimen is sterile. "s.n." means: unnumbered specimens.

1. **TRISTICHA** Du Petit-Thouars, nom. conserv. propos.

Moss-like coenobia, widely differing in shape and size but always strongly ramified and forming dense mats on rocks in streams. Stems thin, terete. Leaves for the greater part tristichous but the regular arrangement in the first-formed parts sometimes disturbed; the blade membranaceous, either nerveless or one-nerved, obtuse or acute, entire but in older leaves often split into 2 or 3 lobes. Flowers at the end of ordinary shoots or on brachyblasts, at first enclosed between 2 or 3 distinctly larger leaves; perianth well-developed, 3-merous, membranaceous and marcescent, petals free or united at the base; stamen 1, filament slender; anther ovate, introrse, with an elongate connective, pollen globose; ovary 3-celled, consisting of 3 equal carpels, ovoid to subglobose, rounded or attenuate at the base; placenta fleshy; fruit dehiscing with 3 equal valves each provided with 3 ribs; seeds numerous.

Type: *Tristicha trifaria* (Bory ex Willd.) Sprengel
Distribution: Tropical and subtropical America, Africa and Asia. **(See map)**

The genus *Tristicha* was described by Du Petit-Thouars in 1806, but the author failed to mention a species. The first two species, which afterwards were referred to this genus, viz. *T. trifaria* and *T. alternifolia*, were described by Willdenow in 1810 and 1811, under the generic name *Dufourea*. This latter has been used for genera belonging to various families, viz. in 1810 by Acharius for a genus of lichens, in the same year by Willdenow in the *Podostemaceae*, in 1815 by Kunth in the *Convolvulaceae*, and in 1837 by Grenier in the *Caryophyllaceae*, in the latter two families the name has since then been replaced by *Breweria* and *Arenaria* respectively.

In the case of the *Podostemaceae* and *Lichenes* the question of precedence is difficult to decide. For the Podostemaceous genus, however, an older name is available, although the latter is not fully legitimate as no species was indicated. This name, viz. *Tristicha*, has been in use since 1825, and it is therefore proposed to conserve it and to reserve *Dufourea* for the lichenous genus. The type-species of the Podostemaceous genus *Tristicha* is T. trifaria (Bory ex Willd.) Sprengel, described in 1810 as *Dufourea trifaria*, and transferred by Sprengel in 1825 to *Tristicha*.

In 1823 Aug. St-Hilaire described a new species of *Dufourea*, viz. *D. hypnoides*, and this was transferred to *Tristicha* by Sprengel in 1827. Richard (1824) also described a new *Dufourea*, viz. *D. boryi*, but this species is according to Tulasne conspecific with *T. trifaria*. Bongard in 1841 founded a new genus *Philocrena* with a single species, *P. pusilla* but this plant too was recognised by Tulasne as conspecific with a *Tristicha* species, viz. with *T. hypnoides*. Gardner (1847) added *T. bryoides* to the list, but this species was rejected by Tulasne, who recognized it as conspecific with *T. hypnoides*. Liebmann described a genus *Potamobryum* with three species, viz. *P. concinnum*, *P. laxum*, and *P. patulum*, but all these species were reduced by Warming to *T. hypnoides*. *Potamobryum patulum*, however, was reinstated by Johnston (1949), but it was once more reduced to *T. trifaria* by van Royen in 1950.

Weddell in 1873 reduced *T. dregeana* (Presl) Tulasne, to a variety of *T. hypnoides*, distinguishing at the same time the varieties *microcarpa* and *hilarii;* the latter variety had been created in 1849 by Tulasne, but in his monograph of 1852 this author did not maintain it. Two more varieties were described by Weddell, viz., the variety *fontinaloides* and the var. *pulchella*. In his monograph Weddell therefore accepts *T. hypnoides* with five varieties, viz. *hilarii*, *pulchella*, *fontinaloides*, *dregeana*, and *microcarpa*, and as distinct species *T. trifaria* and *T. alternifolia*. *T. bifaria* from the Philippines is mentioned as a dubious species.

Warming in 1901 (p. 24—39) came to the conclusion that *Potamobryum concinnum*, *P. patulum* and *P. laxum* are conspecific with *T. hypnoides*. He united, moreover, *T. trifaria* with *T. hypnoides*, using the epithet *hypnoides* for the combined species. He left therefore but two species in *Tristicha*, viz. *T. alternifolia* and *T. hypnoides*, but as the specific epithet *trifaria* dates from 1810 and *hypnoides* from 1823 it is clear that the correct name is *T. trifaria*. This was independently recognised by Taylor in Andrews in 1950, by van Royen in 1950 and by Horn af Rantzien in 1950 and 1951. The first used the name *T. trifaria* (Bory) Sprengel, the second *T. trifaria* (Willd.) Tul. and the third *T. trifaria* (Bory ex Willd.) Tul. Willdenow expressely states that de Bory had asked him to use for his species, if it should prove to be a new one, the name *Dufourea trifaria*, and to credit the species to him. As part of the description of this new species was prepared by Willdenow, it seems justified to add the latter's name to that of de Bory. The transfer of *Dufourea trifaria* to *Tristicha* had already been effected by Sprengel in 1825, whereas the work of Tulasne dates from 1849. The correct name for this species is therefore: *Tristicha trifaria* (Bory ex Willd.) Sprengel.

In the course of my study I have come to the conviction that *Tristicha alternifolia* (Willd.) Sprengel must be regarded as conspecific with *T. trifaria* as there are numerous intermediate stages between these two species. Horn af Rantzien had reported already in 1948 that he could find no difference between these two species, but he did not actually carry out a fusion. As *Dufourea trifaria* was published

in 1810 and *D. alternifolia* in 1811, the specific epithet of the former has priority over that of the latter.

During the preparation of this publication I received from Prof. TOBLER in St. Gallen (Switzerland), a manuscript in which he proposed a new monotypic genus from Uruguay (*Heterotristicha*, based on *H. schroederi* Tobler, spec. nov. inedit.), differing from *Tristicha* in the shorter styles and the free tepals, the leaves, moreover, being larger although otherwise similar to those of *Tristicha*. As a result of a discussion on this question by letter Prof. TOBLER agreed to withdraw this new genus. The differences are so small that this seems to be indicated. The length of the styles is highly variable in *T. trifaria*. As regard the free tepals, I believe that we have in the perianth of this specimen merely an extreme variant of the normally trifid to tripartite condition. The same kind of variability is found in *Weddellina*, where the perianth may be 5-fid to 5-partite or split into 5 free petals. The size of the leaves is so strongly variable that it can not be used for the distinction of species.

Geography (See map)

The genus is distributed over the tropical parts of America, Africa and Asia. The area of one species, T. trifaria, extends from Central America and the West Indian Islands over South America to Africa and Madagascar and the islands to the east of the latter. The second species, T. ramossissima, is found in India and Ceylon only.

1. **Tristicha trifaria** (Bory ex Willd.) Sprengel (1825) 22; Steudel (1841) 715 — *Dufourea trifaria* Bory ex Willd. (1811) 63—64; Steudel (1821) 287; Steudel (1841) 533 — *Tristicha trifaria* (Willd.) Tul., v. Royen (1950) 125—126, f. 54 — *Tristicha trifaria* (Bory) Sprengel, Andrews (1950) 83 — *T. trifaria* (Bory ex Willd.) Tul., Horn af Rantzien (1951) 376—378 — *T. hypnoides* (St-Hil.) Sprengel (1827) 10; Steudel (1841) 715; Nash (1905) 3; Herter (1930) 65; Standley (1937) 472; Dugand (1944) 30; Horn af Rantzien (1948) 186—187, 1 fg. — *T. hypnoides* Sprengel (1841) 715; Weddell (1873) 44—45; Cario (1881) 25, 41, 73, t. 1—24; Warming (1881) 1; idem (EP 1891) 18; idem (1899) 107—114, f. 1—6; idem (1901) 24—30, f. 20—30; Went (1919) 67; Engler (1930) 35—36; Yuncker (1940) 292 — *T. hypnoides* Tul. (1849) 112; idem (1852) 186—189, t. 10 f. 4; Walpers (1852) 443; idem (1858) 791; Tul. (1863) 272—273; Tobler (1933) 299, f. 15 — *T. hypnoides*, Baillon (1888) 257, 267, f. 315, 316; Marie-Victorin & Leon (1944) 351 — *Dufourea hypnoides* St-Hil. (1823) 472; idem (1824) 83; Richard (1829) 636 — *Tristicha bryoides* Gardner (1847) 178; idem (1850) 39; Wight (1852) 35, t. 1920 — *T. dregeana* (Presl) Tul., (1852) 184—185 — *Podostemum dregeanum* Presl (1844) 149 — *Tristicha fontinaloides* Welw. msc. — *Philocrena pusilla* Bongard (1835) 81—82, t. 6; Steudel (1841) 320 — *Potamobryum concinnum*, *P. laxum* et *P. patulum* Liebmann (1849) 513—515 — *Tristicha concinnum* (Liebmann) I. M. Johnston (1949) 130 — *T. hypnoides* (St-Hil.) Sprengel var. *hilarii* Tul., var. *microcarpa* Tul., var. *pulchella* Wedd., var. *fontinaloides* Wedd., var. *dregeana* (Presl) Tul. (1849) 112; idem (1852) 189; Weddell (1873) 45; Engler (EP 1930) 36 — *Dufourea boryi* A. Rich. (1829) 636 — *Cryptocarya tristicha* Taylor msc, in Kew Herbarium — *Tristicha alternifolia* Tul., (1849) 111; idem (1852) 182—183; Steudel (1841) 715 — *Dufourea alternifolia* Willd. (1811) 64; Weddell (1873) 45—46[1]).

Coenobia attached to the substrate by means of branched roots and forming dense but low mats; stems terete, 0.2—10 cm high; leaves for the greater part tristichous and the orthostichies clearly recognizable, one orthostichy on the upper side of the stem, usually consisting of ovate, about 0.4 × 0.5 mm large leaves, the other two orthostichies at the flanks, at the same level, usually consisting of obliquely inserted, spatulate, about 0.2 × 2 mm large leaves; on the first-formed parts the regular distribution is disturbed and then the leaves appear in irregular whorls of 3 to 6; all leaves nerveless or with a distinct nerve, entire, obtuse to acute, sessile, usually of two kinds, sometimes all similar, mature leaves split into 2—4 acute lobes. Fertile branches with a terminal flower originally enclosed within 2 or 3 membranaceous, 2—2.5 mm long leaves; perianth trifid to tripartite or with three free petals; lobes obtuse, with a distinct nerve, membranaceous, 1—2 mm long; stamen 1, from 1.5—2.5 mm long; anther sagittate or ovate, about 0.8 mm long, truncate; top sometimes darkly coloured;

[1] The literature dealing with T. alternifolia is not completely given.

base of thecae obtuse; pollen grains globose 17 μ in diam; ovary ellipsoidal, rounded or attenuate at the base, 0.5—1 mm long, about 0.8 mm in diam; styles 3, linear, about 0.5 mm long. Fruit dehiscing into 3 valves; each valve with 3 ribs; pedicel 3—20 mm long.

Type: Du Petit-Thouars s.n., P; collected in Madagascar.

Distribution: From Mexico to Madagascar.

Vernacular names: pesacarne (Panama, Costa Rica), paste de piedra rio (Guatemala).

Use: According to STANDLEY it is eaten by cattle in the dry season.

Mexico: Rio San Francisco, Barranca falls, Liebmann s.n., fl.fr. July (C); between Asleton and Maloapan, Liebmann s.n., May (C); Cordita, Gray s.n., fl. May (GH); Cordoba, dept. Vera Cruz, Greenman 124, fl.fr. Jan. (F, GH, MO); Rio Quiotepec, dept. Oaxaca, Conzatti 3914, March (US); prov. Orizaba, Schaffner s.n., fl. (P); idem, Weber s.n., (P); without loc., Sumichrast s.n., fl.fr. (P).

Honduras: Vicinity of Siguatepeque, dept. Comayagua, Standley 55915, fl.fr. Febr. (F); 56283, fl. fr. Febr. (F); 56284, fl. fr. Febr. (F, US); Mt Cangrajal, Yuncker c.s. 8853, fl. Aug. (MO); Uyuca, dept. Morazán, Rodriguez 1597, fl. fr. Nov. (F); Rio Yeguare, dept. Morazán, Rodriguez 2552, March (F); Rio Caparrosa, Zamorano, dept. Morazán, Rodriguez 3663, fl. fr. Jan. (F); Rio Yeguare, Standley 1123, fl. fr. Nov. (F); idem, Standley 1696, fl. fr. Jan. (F); vicinity of Comayagua, Rio Humuya, Standley & Chacon 5604, fl. fr. March (F); vicinity of Siguatepequez, dept. Comayagua, Standley & Chacon 6743, March (F); Rio Yeguare, near El Zamorano, Standley & Molina 4660, fl. fr. Febr. (F); Rio San Alejo, dept. Atlántida, Standley 8004, April (F); vicinity of El Zamorano, Standley 3990, Febr.—March (F).

Nicaragua: Rio Pais, Ørsted s.n., fl. fr. Febr. (C); Salorina (?), Ørsted s.n., fl. fr. (C); Nicaragua lake, Ørsted s.n., fl. fr. (C); idem, Wright s.n., fl. fr. (GH, MO, NY, P, US); Rio d'Oro, Ørsted s.n., fr. (C); Libertad, dept. Chontales, Rio Mico, Standley 8936, fr. May—June (F); Jinotega, dept. Jinotega, Standley 9662, June—July (F).

Guatemala: Mazatenango, Bernouilli 39, fl. fr. Nov. (BR, NY); Retalhuleu, Kellerman 6662, fl. fr. Jan. (F); idem, Standley 88817, fl. fr. Febr. (F); between Retalhuleu and Asintal, Standley 87867, fl. fr. Febr. (F); Rio Bobos, Quebrados, Blake 7542, fl. fr. May (GH, US); Rio Lima, Muenscher 12033, May (F, GH); Rio Siguacan, Muenscher 12030, fl. May (F, GH); near Los Verdes, dept. Santa Rosa, Standley 60408, fl. fr. Dec. (F); Rio Pinule, Steyermark 32914, fl. fr. Dec. (F); between Finca Pirineos and Soledad, dept. Quezaltenango, Steyermark 33576, fl. Jan. (F); Quebrada San Géronimo, Steyermark 33363, 33368, Jan. (F); Rio Juyamá, Steyermark 39119, fl. fr. April (F); Rio Samalá, Steyermark 34548, fl. fr. Jan. (F); hills between Jutiapa and Plan de Urrutia, Standley 75497, Oct. (F); idem, Standley 88363, fl. fr. Febr. (F); near Las Delicias, Standley 88000, 88002, fl. fr. Febr. (F); Ajaxá, Standley 88248, fl. fr. Febr. (F); Rio Vil, Standley 88311, fl. fr. Febr. (F); vicinity of Chiquimulilla, Standley 79714, Nov.—Dec. (F); along Avellana-road, Standley 79471, fl. fr. Dec. (F); near Guazacapán, Standley 78604, fl. Dec. (F); Rio Sébol, Steyermark 46314, May (F); Rio Teculutan, Steyermark 42113, fl. fr. Jan. (F); La Puenta, Wendland s.n., fl. Dec. (W).

El Salvador: vicinity of Ahuachapán, Standley & Padilla 2595, Jan. (F); idem, Standley & Padilla 2763, fl. fr. Jan. (F); idem, Standley & Padilla 2784, fl. fr. Jan. (F); vicinity of San Vicente, Standley & Padilla 3827, Febr. (F); dept. Sta. Anna, above Hacienda San José, NW of Metapán, Fassett 28763, Febr. (U, WI); dept. Sonsonate, between Nahuizalco and Juayúa, NW of Sonsonate, Fassett 28730, Jan. (U, WI); dept. San Salvador, Las Cataractas, Panchimalco, Fassett 28668, fr. Jan. (U, WI); idem, Fassett 28670, Jan. (U, WI); idem, Fassett 28667, Jan. (U, WI); idem, Fassett 28665, Jan. (U, WI).

Costa Rica: Aquacaliente, Pittier 892, fl. fr. Febr. (BR, P, US); Bornca, prov. San José, Pittier 3845 & 659 (BR); Rio Ceibo, Pittier 3847, (BR); Rio Torres, prov. San José, Tonduz 7126, fl. fr. Jan. (GH, MO, NY, US); idem, Tonduz 11294, July (P, US); Quebrada Azul, prov. Guanacaste, Standley & Valerio 46346, fr.

Jan. (F); vicinity of Tilarán, prov. Guanacaste, Standley & Valerio 45033, fl. fr. Jan. (F); idem 45031, Jan. (F, US); idem 44263, Jan. (F); idem 45684, fl. fr. Jan. (F, US); idem 46656, fl. fr. Jan. (US); idem 46615, fl. fr. Jan. (F); idem 46633, fl. fr. Jan. (F); Rio de Las Piedras, prov. Tilarán, Alfaro 111 & 111,2, fl. fr. March (F); idem, Alfaro 112, fl. fr. March (F); Quebrada Tronadora, prov. Tilarán, Alfaro 113, fl. fr. March; idem, Alfaro 125 & 125.A, fl. fr. April (F); San Sebastian near San José, prov. San José, Standley 49291, fl. fr. Febr. (F); vicinity of El General, prov. San José, Skutch 2522, fl. fr. Jan. (NY, S, US); idem, Skutch 2473, fl. fr. Jan. (GH, NY, US); Rio Jorco, Valerio 1130, fl. (NY, US); Siguirres, Alfaro 104, fl. fr. Febr. (F); Rio Santa Rosa, Alfaro 114, fl. fr. March (F); vicinity of Pejivalle, prov. Cartago, Skutch 4603, fl. fr. Jan. (F, GH, MO, NY, US); San Antonio de Bilar, Echeverria 350, March (F); Rio Agua Caliente, prov. Cartago, Torres 3, (F); St. Anna, Wendland s.n., fl. April (W); without loc., Tonduz 9838, (G.-Boiss); idem, Pittier & Tonduz s.n. (BR).

Panama: Penonome, Williams 1043, fl. fr. Febr. (NY); Rio Paraiso, Standley 29878, fl. fr. Jan. (C, S, US); Rio Pedro Miguel, Standley 29933, Jan. (US); idem, Standley 29934, fl. fr. Jan. (GH, US); Quebrada Ancha, Steyermark & Allen 17130, fl. Dec. (MO); Las Cascades, Dodge & Hunter s.n., fl. Dec. (MO); Rio Boqueron, Steyermark & Allen 17257, fl. Dec. (MO, S, U); Rio Indio, Steyermark & Allen 17400, fl. Jan. (MO); Rio Anton, Muenscher 16264, March (MO, U).

Colombia: Jabano, André K 1595, (F, NY); Jocola, Rio Dagua, André K 2650, April (F, NY); Rio Mayo, André 2918, (F); La Union André K 1597, (F, NY); Paramo de Guapascal, André K 1596, (F, NY); Salto de Tequendama, Killip 34039, fl. Febr. (F, GH, MO, US); Rio Rancheria, Haught 4009, Febr. (US).

Cuba: Prov. Pinar del Rio:—Rio San Sebastian, Ekman 13797, fl. May (MO, S); idem, Ekman 18054, Nov. (F, G.-Del., GH, NY, P, S, US); Rio Guao, Britton c.s. 9638, fl. fr. Dec. (F, GH, NY, US); Rio Mestanza, Britton c.s. 10162, fl. March (NY); Rio Portales, Ekman 18700, fl. March (S); without loc., Wright 3195, fl. fr. (GH, MO, NY, P, S, W).

Venezuela: Near Valencia, Einar Staal s.n., (C).

British Guiana: Puruni river, Jenman 7607, fl. fr. Oct. (C, NY, U); without loc., Schomburgk 432, fr. (C, L, P, W); idem, Schomburgk 431, (G.-Del., L, P, W).

Brazil: Prov. Goyaz — Rio Tocantin, Weddell 2366, fl. fr. Febr. (P); Rio Bacalhao, Glaziou 22005, July (BR, C, G.-Boiss., G.-Del., P, S); idem, Glaziou 22002, fl. fr. July (BR, C, G.-Del., P, S); idem, Glaziou 21999, fl. July (BR, C, G.-Del., P, S); idem, Glaziou 22010, fl. July (BR); Rio Vermelho, Weddell s.n., fl. fr. Febr. (P); idem, Glaziou 22007, fl. fr. July (C, G.-Boiss., G.-Del., P, S); idem, Glaziou 22000, fl. fr. July (BR, C, G.-Del., P, S); Rio Douradinha, Glaziou 21995, fl. fr. Aug. (BR, C, G.-Del., GH, P, S); Rio Babylonia, Glaziou 21996, Aug. (BR, C, F, G.-Del., P, S); Rio Macaco, Glaziou 22004, fr. July (F, G.-Boiss., G.-Del.); Rio Trinidade, Glaziou 21993, fr. Aug. (BR, C, G.-Del., P, S) — Prov. Rio de Janeiro — Rio Negro, Ronca Cao rapids, Glaziou 13140, fl. fr. June (BR, C, G.-Del., P, US); idem, Glaziou s.n., fl. July (C); idem, Glaziou s.n., fl. fr. June (C); Nova Friburgo, Schwacke 3032, fl. fr. Jan. (C); without loc., Glaziou 15442 (C, P) — Prov. Sao Paulo — Rio Piracicaba, Glaziou 19817, (C, P); idem, Glaziou 19816, (C, P); idem, Glaziou 15443, (P); idem, Glaziou 16358, (P); Rio Jaguary, Mosén 4587, Jan. (P, S) — Prov. Sta. Catharina — Blumenau, Rio Itayahy, Schwacke 5011, (C); idem, Muller 2, (C); Rio Capinzal, Dusén 17871, fl. Febr. (S); without loc., Schenck 327, Sept. (C) — Prov. Pernambuco — near Tapera, Bento Pickel 1420, fl. fr. Nov. (GH, US); Rio Pinga, v. Luetzelburg 15512 & 16032, Sept. (M) — Prov. Para — Rio Uaupès, Spruce 1084, fl. fr. Sept. (C, GH, NY, P); near Santarem, Spruce s.n., fl. fr. Sept. (C, G.-Del., P, W) — Prov. Ceara — Acude Acaraque, Drouet 2735, Sept. (F, GH, NY, S, US); Rio Salgado, Gardner 1844, fr. (BR, C, G.-Del., L, P, W) — Prov. Matto Grosso — Cusiem, near Palmeiras, Lindman A 2451, fl. Dec. (S) — Prov. Rio Grande do Sul — Silveira Martius, Arroio Grande, Lindman A 1235, fl. March — without known province — Rio Tieté, Riedel 1093 (GH); Rio Iguapé, Iporanga, Puiggari 279, fl. July (G.-Del.)

Uruguay: Salto Grande, Uruguay-river, Ostén 2902, fl. fr. Dec. (C); idem, Berro 5154, 5156, 5162, 5167, fl. fr. April (C); Rio Negro, Salto de Sierra, Ostén 18758, Jan. (F, S); idem, Berro, 1, 4, 5165, fr. March (C); Barra de Vera, Berro

3393, Febr. (C); idem, Berro 5155, 5163, 5164, Febr. (C); Salto Chico, Berro 5169, fr. April (C); Barra del Tala, Berro 5166, Febr. (C).

Argentina: According to Horn af Rantzien (1951) this species is reported from the Territory of Missiones.

Without known state: Truando-falls, Schott 40862, 40875, 40882, fl. Jan. (F); idem, Schott 40861, fl. fr. (F, NY).

2. **WEDDELLINA** Tul.

Medium-sized to large coenobia consisting of branched sterile shoots and unbranched fertile shoots springing from the same slightly flattened and branched root. Fertile shoots small to medium-sized provided with a few squamiform leaves and ending in a single flower. Flower when young enveloped by squamiform leaves; tepals 5, distinct, membranaceous, free or slightly united at the base, provided with a single distinct nerve; stamens 5—25, in a single complete whorl; anthers dehiscing introrsely; pollen grains ellipsoidal, 3-colpate; ovary 6-ribbed, 2-celled, the cells equal; style 1, filiform, discoidally flattened at the top. Fruit opening with 2 equal valves; each valve provided with 3 indistinct ribs. Sterile shoots repeatedly forked, densely covered with 2- to 6-dentate scales and carrying small alternate leaves; the leaves repeatedly forked and covered with scales; ultimate divisions with tufts of minute filaments in the axil of 1- to 6-dentate scales.

Type: *Weddellina squamulosa* Tul.

Distribution: 1 species in British Guiana, Suriname, Colombia and Northern Brazil (**See map**)

1. **Weddellina squamulosa** Tul., forma **squamulosa**, Tul. (1849) 113—114; idem (1852) 195—197, t. 13 f. 5; Walpers (1852) 444; idem (1858) 792; Weddell (1873) 48; Goebel (1893) 349—350, t. 31; Warming (1894) 18, f. 13; Wächter (1897) 382—397, f. 9—21; Warming (1901) 53; Engler (1930) 31—32, f. 16 B, C, f. 23; v. Royen (1948) 382.

Small to large coenobia with the shoots arising from about 1 mm wide roots. Fertile shoots 2—12 cm long, at the base indistinctly, towards the top more distinctly winged, the wings passing into the nerves of the tepals; leaves numerous, spirally arranged, squamiform, cordate to obovate, sessile, subamplexicaul, obtuse to acute, 1—2 mm long and about 1 mm wide. Flowers pink to lilac or white; tepals spathulate, entire, obtuse or mucronate, crested at the base, free or shortly united, imbricate, 3—6 mm long; stamens 5—15, from 3.5—6.5 mm long; anthers 0.5—1 mm long, obtuse to rather deeply emarginate at the top, and with a cordate base; lobes obtuse; pollen grains 15 μ high, 10 μ diam; ovary ellipsoidal to subglobose, obtuse, attenuate at the base, 2.5—4 mm high, 1.5—2 mm diam; style 0.5—1 mm long. Sterile shoots 2.5—80 cm long, terete, slightly compressed at the base.

Type: Schomburgk 433, K, duplicates BR, C, G.-Del., K, L, P, US, W; collected in British Guiana.

Distribution: Colombia, British Guiana, Suriname, Northern Brazil.

Colombia: Rio Uaupès, Mindu rapids, Schultes s.n., fl. Jan. (GH); idem, Yuruparifalls, Cuatrecasas 6986, Sept. (US); idem, Allen 3214, fl. Nov. (MO, U).
British Guiana: without loc., Schomburgk 433, fl. fr. (BR, C, G.-Del., K, L, P, US, W); Essequibo-river, Head-falls, A. C. Smith 2103, fl. fr. Oct. (F, G.-Del., MO, NY, S, U, US); Mazaruni-river, Caburi-falls, Jenman 7721, fl. fr. Oct. (C, NY, U); idem, without loc., Goebel s.n. (ex mss); Potaro-river, Amatuk-falls, Jenman 7421, fr. Oct. (NY, U); idem, Jenman 7493, (S); idem, Pakatuk-falls, Jenman 7740, fl. Oct. (U); idem, near Cobanatuk ,Jenman 7418, fl. Oct. (F); idem, Jenman 7420, fl. Oct. (B); idem, Tumatumari-falls, Linder 14, fl. fr. Sept. (GH, NY); idem, Jenman 936, fl. fr. Sept. (F); Puruni-river, Waramboo-falls, Jenman 7604, fl. fr. Oct. (NY, U); idem, Thomas-falls, Jenman 7609, fl. fr. Oct. (U); idem, idem, Jenman 7710, fl. fr. Oct. (NY, U); Demerara, Jenman 1099, fl. fr. Jan. (P); Mazaruni-river, Jenman s.n. (NY).
Suriname: Saramacca-river, Gran Dam, Maguire 24925, fl. fr. Oct. (F, NY, U, US).
Brazil: Rio Oyapock, Grande Roche, v. Luetzelburg 21662, fl. July (M); idem, Salto Crignon, v. Luetzelburg 20246, July (NY, W); Rio Uaupès, v. Luetzelburg 23153, 23171, 23180, 23188, 23216, 23250, 23254, 23258, 23273, 23284, 23292, fl. Nov. (M); without loc., Huber 1817, (G.-Boiss., W); Rio Pauri, v. Luetzelburg 23159, 23210, fl. Nov. & Dec. (M).

Forma **uaupènsis** (Benth. & Hooker) v. Royen, nov. comb. — *Weddellina uaupensis* Benth. & Hooker (1880) 109; Engler (1930) 32.

Flowers similar to those of forma squamulosa but provided with 20—25 stamens. Otherwise similar to forma squamulosa.
Type: Spruce 2752 in K, duplicates in BR, C, GH, K, NY, P; collected in Brazil.
Distribution: Brazil.
Rio Uaupès, near Panurè, Spruce 2752, fl. fr. Oct.

3. **MOURERA** Aublet

Small to very large herbs, stemless or with a short stem formed by the fusion of the leaf-bases; leaves radical, distichous, small to very large, elliptical and then with a strongly fimbriate margin, cuneate, pinnatilobed or repeatedly forked with the ultimate divisions filiform, sometimes very coarse and provided with many rigid outgrowths at the upper surface; the elliptic, cuneate and pinnatilobed leaves distinctly nerved. Flowers in 2-sided spiciform monochasia, the monochasia spring from the axils of the upper leaves and are either pedunculate or sessile, few- to many-flowered, branched or unbranched, sometimes very short or reduced to a single flower. Flowers alternating with decurrent bracts; the latter on both sides with a distinct wing, the basal margins of which cover the apical margins of the preceding bracts. Juvenile spathella clavate, obtuse or acute; mature one infundibuliform, exceeding the bracts; tepals 5—20, free, in a single complete whorl; stamens 5—35 in an incomplete or in one or two complete whorls; filaments lanceolate or linear to elliptical; anthers narrow, with deeply incised base, dehiscing introrsely or when inserted in two whorls partly extrorsely; pollen grains ellipsoidal, 3-colpate; ovary

2-celled, ellipsoidal, attenuate at the base, with two equal carpels, 6- to 14-ribbed, but 2 of the ribs sometimes indistinct; styles 2, filiform to spatulate, free or cohering at the base. Fruit with 2 equal valves, each with 3 or 5 ribs; styles, filaments and tepals marcescent.

Type: *Mourera fluviatilis* Aublet.

Distribution: 6 species distributed over Guiana, Venezuela, Colombia, Northern and Central Brazil. **(See map)**

Mourera is distinguishable from the majority of the *Podostemaceae* by the arrangement of its flowers, which are borne in 2-sided spiciform monochasia. It shares this character with *Tulasneantha* [1] and *Lonchostephus*, and these three genera therefore are referred by me (1951, p. 13), to a tribe of their own, the *Mourereae*. However, this character is sometimes indistinct e.g. in *Mourera alcicornis*. Originally this species was described by Tulasne as belonging to *Ligea* and Weddell subsequently inserted it in *Oenone*. A remark made by Spruce on one of his labels shows that he considered the species to belong to *Mourera*. When investigating the material of *Mourera alcicornis* I came to the conclusion that this species is provided with monochasia, although very short ones and that some specimens show a distinct peduncle. When one investigates specimens with very short peduncle, one would be inclined to refer this species in *Oenone*, but when one becomes acquainted with those provided with a distinct peduncle, one does not hesitate to insert it in *Mourera*. The rough upper surface of the leaves also points in the direction of *Mourera*. Therefore I have inserted *Oenone alcicornis* (Tul.) Wedd. in *Mourera* as *M. alcicornis* (Tul.) v. Royen.

Key to the species:

1*a*. Small plants with few-flowered inflorescences provided with a very short peduncle. Leaves elliptical to cuneate, pinnatilobed . . . . . 6. **M. alcicornis** (Tul.) v. Royen.
b. Small to very large plants with distinctly stalked inflorescences. Leaves either repeatedly forked or entire to pinnatisect 2
2*a*. Inflorescences branched 5
b. Inflorescences unbranched 3
3*a*. Leaves repeatedly forked 4
b. Leaves entire to pinnatisect, margin often fimbriate . 1. **M. fluviatilis** Aublet
4*a*. Pinnae of the leaves thick and fleshy and the filiform ultimate divisions numerous . . . . . 3. **M. glazioviana** Warming
b. Pinnae membranaceous and the filiform ultimate divisions few in number . . . . . 4. **M. weddelliana** Tul.
5*a*. Leaves large, entire or provided with a strongly laciniate margin . . . 8
b. Leaves of medium size, i.e. up to 20 cm long, repeatedly forked . . . 6
6*a*. Stamens 7—15, all free 7
b. Stamens 20—25, sometimes a few united . . . . . 2. **M. schwackeana** Warming
7*a*. Pinnae of the leaves thick and fleshy; the filiform ultimate divisions numerous . . . . . 3. **M. glazioviana** Warming
b. Pinnae membranaceous; the filiform ultimate divisions few in number . . . . . 4. **M. weddelliana** Tul.
8*a*. Stamens in a complete whorl . . 5. **M. aspera** (Bong.) Tul. forma **aspera**
b. Stamens in an incomplete whorl . . . . . 5. **M. aspera** (Bong.) Tul. forma **minor** Warming

[1] See for this name: v. Royen, Pod. New. World, part 1 (1951) 9

1. **Mourera fluviatilis** Aublet, 1 (1775) 582—584; idem, 4 (1775) t. 233; Lamarck, Encycl. (1796) 334; idem, Ill. Encycl. (1796) t. 480; St. Hilaire (1805) 576; Tul. (1849) 93; idem, Tul. (1852) 62—65, t. 1 f. 5; Walpers (1852) 432; Walpers (1858) 775; Weddell (1873) 49—50; Warming (EP 1891) 20; Pulle (1906) 194; Matthiesen (1908) 20—26, t. 8, f. 62—67, t. 9 f. 68—79, 87; Glaziou (1911) 574; Went (1912) 51—66, t. 1 f. 11—16, t. 14/15 f. 123—173; Engler (1930) 42; Graham (1933) 140; v. Royen (1948) 383 — *Lacis fluviatilis* Schreber (1789) 366; Willdenow (1797) no. 1225; Persoon (1807) 81; Steudel (1821) 460; Martius & Zuccarini (1824) 6; Steudel (1841) 2; Bongard (1835) 73; v. Chamisso (1835) 503 — *Stengelia* Necker (1790) 258, no. 1011.

Large herb, either stemless or provided with an unbranched, 1—5 cm long and 0.5—1.5 cm thick stem; leaves of different size and shape, 8—200 cm long, 2—30 cm wide, pinnatinerved; nerves prominent underneath; secondary nerves anastomosing, fluctuating; upper surface of the leaf rough; lower surface glabrous. Inflorescences 5—60 cm long, 3—8 m wide, unbranched, very rarely terminated by a 2 cm long leaf; bracts boat-shaped, slightly crested, rough, 5—13 mm long; pedicel of the flower 1—4 cm long, widened at the top; juvenile spathella obtuse or acute; mature one cupuliform to tubuliform, 10—15 mm long. Flowers pink to violet; tepals 16—20, squamiform and lanceolate, 0.5 mm long or less; stamens 20—35, from 6—12 mm long, in one or two whorls; anthers 3—4 mm long, with one or two acute teeth at the top; base cordate; in the inner whorl dehiscing extrorsely, in the outer whorl dehiscing introrsely, pollen grains 17 μ high, 16 μ diam; ovary 5—12 mm long, 2—3 mm diam, with 6 or 8 ribs; juvenile styles spoonshaped; mature ones filiform, 1.5—2.5 mm long. Fruit similar to the ovary; each valve with 3 or 5 ribs, 8—13 mm high; pedicel 1—4 cm long.

Type: Aublet s.n., P; duplicates BM, MO.

Distribution: Venezuela, Guiana, Northern Brazil.

Vernacular names: mourerou (French Guiana), koemaroe njam njam (Suriname), arapsoe-banja (Suriname), paco (British Guiana), avenca d'agua (Brazil).

French Guiana: Sinemarie-river, Aublet s.n., fl. fr. (BM, MO, P); without loc., Leprieur 1834, fr. (G.-Del.); idem, Martin 135, fl. fr. (P); idem, Martin s.n., fr. (B, C); idem, Rothery 55, fr. (K).

Suriname: Suriname-river, Wullschlaegel 1673, fr. (GOTT., U, W); idem, Hostmann 1248, fr. (FI, G.-Del., GH, K, U, W); Upper Nickerie-river, Tulleken 438, Sept. (L, U); Marowyne-river, Kappler & Hohenacker s.n., fl. fr. Aug. (S); idem, Armina-falls, Went 462, fr. Oct. (P, U); idem, near Poeloegoedoe, Versteeg 610, July (U); idem, Gonsoetoe-falls, Florschütz 542, fr. Dec. (U); Coppename-river, Raleigh-falls, Boon 1246, fr. Oct. (U); idem, Boon 1030, fl. fr. Aug. (U); idem, Boon 1079, fl. fr. Aug. (U); idem, Boon 1141, fl. fr. Sept. (U); idem, Lanjouw 964, fl. fr. Sept. (U); Gonini-river, BoneDoro-falls, Versteeg 46, fl. fr. Aug. (U); idem, Versteeg 150, fl. fr. Aug. (U); Tapanahoni-river, Versteeg 749, Aug. (U); idem, near Drietabbetje, Florschütz 510, fl. fr. Dec. (U); idem, Zandkreek soela, Geyskes 962, fl. fr. Sept. (U); Corantyne-river, Rombouts 188, fl. fr. Sept. (U); Saramacca-river, Kwattahede to Tukoesoe, Maguire 23955, fl. fr. June (U); idem,

Maguire 24922, fl. fr. Oct. (F, K, NY, U, US); idem, near Pakka-pakka, Maguire 24949, fl. fr. Oct. (F, K, NY, U, US); idem, Saracreek near Dam, Florschütz 167, fl. fr. Nov. (U); without loc., Tresling 81, (U); Tresling 101, fl. fr. (U); Went s.n. (U).

British Guiana: Demerara-river, Jenman 1151, fl. fr. (K, P); idem, Parker s.n., fl. fr. (K); New River, Weber s.n., Aug. (GH); idem, King Williams falls, Anderson 748, fl. fr. (K); Puruni-river, Big Falls, Jenman 7610, fl. fr. Oct. (BM, C, F, NY, P, U); Essequibo-river, Twasinki-falls, A. C. Smith 2139, fl. fr. Sept. (F, G.-Del., K, MO, NY, S, U, US); idem, near mouth of the Onorocreek, A. C. Smith 2648, fl. fr. Dec. (F, G.-Del., K, MO, NY, S, U, US); idem, Kurupucari-falls, N. Beccari 150, fl. fr. Nov. (FI); Rupununi-river, Myers 5593, fl. fr. Nov. (K); Cuyuni-river, Akaio-falls, Sandwith 695, fl. fr. Nov. (K); idem, Tutin 27, fl. fr. May (BM); Kataboo region, Graham s.n. (U); Takutu, Appun 1637, fl. fr. (K); Cabalebo-falls, Im Thurn s.n., fl. fr. Oct. (K); without loc., Schomburgk 295, fl. fr. (BR, C, CGE, F, FI, G.-Del., GH, K, L, P, W); idem, Schomburgk 351, fl. fr. (BM, G.-Del., P, W); idem, Schomburgk 33 (P).

Venezuela: Alto Caura, Raudal, Bucadesode, Cardona 105, fl. fr. March (US); Rio Revaloso, Othmer s.n. (ex mss Matthiesen).

Brazil: Alto Cunnony, Goeldi 1154, fr. (F, G.-Boiss); Rio Oyapock, Grande Roche, v. Luetzelburg 20258, fl. July (M, W); idem, Salto Manôa, v. Luetzelburg 20324, fl. fr. July (M); idem, idem, v. Luetzelburg 20241, fl. July (M); idem, idem, v. Luetzelburg s.n., fr. July (M); idem, Salto Caxira, v. Luetzelburg 21655, March (M); Rio Ariramba, Ducke 14891, fl. fr. Oct. (CGE); Rio Branco, Kühlmann 162, fl. fr. Febr. (CGE); idem, Ule 7966, fr. Jan. (K); Rio Trombetas, Porteira rapids, Traill 802, March (K); without loc., Gardner 1848 (W); idem, Huber 1790 (G.-Boiss., W).

2. **Mourera schwackeana** Warming (1899) 117—118, f. 10; Engler (1930) 42.

Small to medium-sized herb with an unbranched, compressed, 0.5—1.5 cm long stem. Leaves 2 or 3 times forked, 5—18 cm long; petiole cuneate, with two wings at the base, amplexicaul and decurrent, 1—5 cm long; ultimate divisions filiform, 4—7 mm long. Inflorescence branched, 1.5—5 cm long, at the ramifications with narrow, about 5 mm long leaves; common peduncle terete, densely covered with coarse papillae, slightly winged at the top, 1—2 cm long; the spiciform monochasia 0.6—3 cm long. Flowers few; pedicel 0.5—5 cm long; juvenile spathella clavate, obtuse or acuminate, stalked; mature spathella infundibuliform, 4—12 mm long; bracts boat-shaped at both sides, 1.5—8 mm long; tepals 10—15, linear, up to 0.5 mm long, sometimes a few united; stamens 20—25, in 2 whorls, 3—6.5 mm long, anthers 1—1.5 mm long, acute, cordate at the base; base of thecae acute to obtuse, those of the inner whorl dehiscing extrorsely, those of the outer one introrsely; pollen grains 19 μ high, 12 μ diam; ovary obtuse to acute, 3—7 mm high, 1.5—2 mm diam, with 6 ribs; styles spatulate or obovate, obtuse, subpapillate, about 1 mm long. Fruit similar to the ovary.

Type: Schwacke 4986, C; duplicates P, U; collected in Brazil.

Distribution: Brazil.

Prov. Piauhy, Schwacke 4986, fl. fr. (C, P, U); prov. Ceara, Glaziou 15444 d, fl. fr. (C, P).

3. **Mourera glazioviana** Warming (1899) 114—117, f. 7–9.

Small to medium-sized herb; stem terete, 0.5—1 cm long. Leaves 3 to 4 times forked, 3—6 cm long, 2—6 cm wide; petiole fleshy, subcompressed, 1—4 cm long, about 1 cm in diam, the filiform ultimate divisions numerous. Inflorescences branched or unbranched, 2—14 cm long; common peduncle twisted, sometimes flexuose; the rachis 2—10 cm long, provided with linear, 1—3 cm long leaves, and compressed, widened and winged below the spiciform monochasia, densely covered with coarse papillae; bracts 0.5—7 mm long; narrowly sulcate; flowers loosely scattered; pedicel 0.3—3 cm long; juvenile spathella obtuse, stalked; mature one infundibuliform, 4—7 mm long; tepals 7 or 8, linear, acute, 0.5 mm long or less; stamens 7 or 8, from 3 to 5 mm long; anthers obtuse or acute, 1.5—2 mm long, base sagittate; pollen grains incompletely known; ovary obtuse, 2.5—5 mm long, 1—1.5 mm diam, with 6 ribs; styles spatulate, subpapillate, free, about 0.5 mm long. Fruit similar to the ovary.

Type: Glaziou 21984 a, C, duplicate P; collected in Brazil.

Distribution: Known from the type-collection only.

Rio Urucuya, Glaziou 21984 a, fl. fr. June (C, P).

4. **Mourera weddelliana** Tul., (1849) 93; idem (1852) 66—68, t. 1 f. 4; Walpers (1852) 433; idem (1858) 776; Weddell (1873) 50–51; Baillon (1888) f. 319, 320; Warming (EP 1891) 20, f. 11; Engler (1930) 42, f. 14.

Small to medium-sized herb; stem unbranched, 0.5—1 cm long. Leaves 2 to 7 times forked, antler-like, 5—15 cm long; petiole compressed, 2–7 cm long, widened at the base; the filiform ultimate divisions few in number. Inflorescences usually branched, 2.5—15 cm long; at the ramifications with 0.5—4 cm long, bract-like leaves; common peduncle terete, slightly winged, densely covered with rough papillae, 1.5–8 cm long. Flowers numerous to very few; pedicel 1—12 cm long; juvenile spathella clavate, stalked, nippel-shaped at the top; mature spathella infundibuliform, 4—15 mm long; bracts boat-shaped at both sides, acute, 3—10 mm long; margins membranaceous; the basal margin decurrent along the rachis over a long distance; tepals 9—12, linear, acute, up to 0.5 mm long; stamens 10—15, in one or two whorls, 4–7 mm long; anthers 1.5–2 mm long, acute, with one or two tips; base of the thecae acute; pollen grains 16 μ high, 14 μ diam; ovary acute to obtuse, 4—5 mm high, 1.5—2 mm diam, with 6 prominulous ribs; styles filiform, subpapillate, free, 1—1.5 mm long. Fruit similar to the ovary.

Type: Weddell 2320, P, duplicates C, F, K, NY; collected in Brazil.

Distribution: Brazil.

Rio Tocantin, prov. Para, Weddell 2320, fl. fr. June (C, F, K, NY, P); without collector, Sta. Catharina, fl. fr. (P).

5. **Mourera aspera** (Bong.) Tul. forma **aspera**, Tul. (1849) 93; idem (1852) 65—66; Walpers (1852) 432; idem (1858) 776; Tul. (1863) 236; Weddell (1873) 50; Warming (1888) 493—503, t. 26 f. 1—6, t. 27 f. 1—23; Warming (EP 1891) 20, f. 12; Glaziou (1911) 574; Tobler (1933) 289—295, f. 1—10; Steude (1935) 627—650, f. 1—18; Engler (1930) 42, f. 15 — *Lacis aspera* Bongard (1835) 73—74, t. 2.

Medium-sized to large herb, stemless or with a short up to 6 cm long and 1 cm thick stem. Leaves of different shape and size 10—30 cm long, 4—14 cm wide [1], entire or the margin strongly lacerate, pinnatinerved; nerves at both sides prominent; upper surface provided with folds running nearly rectangular to the primary nerves, rough, at the lower side visible as grooves; underside glabrous. Inflorescences branched or unbranched, 7—24 cm long, with bract-like 3—9 cm long, 0.5—5 cm wide leaves at the ramifications; common peduncle quadrangular, twisted, compressed, sometimes slightly winged; monochasia 0.5—5 cm long; flowers numerous; pedicels 1—3 cm long; juvenile spathella clavate, obtuse, sometimes mucronate; mature spathella infundibuliform, about 1 cm long; bracts boat-shaped, obtuse, 3—5 mm long; tepals 5—10, either subulate and acute or ovate and provided with 2 tops, 1—2 mm long; stamens 5—10, from 5 to 8 mm long; anthers obtuse or emarginate, 1.5—2.5 mm long; connective slightly protruding; pollen grains 17.5 μ high, 10.5 μ diam; ovary ovoid, 2—6 mm high, 1.5—3 mm diam, rounded to attenuate at the base, 8- to 14-ribbed; styles filiform; juvenile ones emarginate; mature ones obtuse; free or slightly cohering, 2—4.5 mm long. Fruit similar to the ovary.

Type: Riedel 413, LE—I, duplicates F, GH, L, P, S, U, W.

Distribution: Southeastern Brazil.

Vernacular name: golfo de fondo (Bahia).

Use: According to Curran an excellent fishfood.

Prov. Minas Geraes — Rio Arassuahy, Glaziou 13136, fl. fr. June (C, G.-Del., K, P, US); without loc., Mello & Netto 14610, fr. June (US) — Prov. Goyaz — Cascade de Rasgaõ, Glaziou 21983 (BR, C, G.-Del., GH, K) — Prov. Mato Grosso — Rio Arinos, Kühlmann 286, fr. Dec. (SP) — Prov. Sao Paulo — Cachoeira do Marimbondo, Gehrt s.n., fl. fr. July (SP); Rio Piracicaba, Piracicaba-falls, Accorsi s.n., fl. fr. (SP) — Prov. Rio de Janeiro — Rio Negro, Glaziou 13134, fl. June (C, P); idem, Ronca Caõ rapids, Glaziou 13139, fl. fr. June (BR, C, G.-Del., K, P) — Prov. Espirito Santo — Rio Guandú, near Baixo Guandú, Tobler s.n., fl. fr. Sept. (BM) — Prov. Bahia — Colonia de Gongugy, Curran 120, fl. fr. Nov. (GH, US); idem, Curran 540 A, fl. Aug. (GH) — Without known province — Rio Tieté, Riedel 413, fl. fr. Aug. (F, GH, L, LE, P, S, U, W); Rio Jequitinhoa, Pohl 3270 (W); Paraiba, Schott s.n. & 5901, fl. fr. (W); Iporanga, Rio Iguapé, Puiggari (?) 3138, fl. July (G.-Del.); between Victoria and Bahia, Humboot 589 (B) — Without locality — Riedel s.n., fl. fr. (K, L, NY, W); Vauthier s.n. (W); Duparquier s.n., fl. fr. (BM).

Dubious specimens — Rio Parahyba (P), without coll.; without loc., Aug. St. Hil. 646 (P).

[1] Tobler (1933) reports that the leaves reach sometimes a width of 1 m.

Forma **minor** Warming (1899) 114.

Differs from the forma aspera in the smaller, suborbicular leaves, in the shorter, up to 10 cm long inflorescences, and in the 4—5 stamens, which are inserted in an incomplete whorl, while the 6 or 7 tepals are inserted in a complete whorl.

Type: Glaziou A, in C, duplicate P; collected in Brazil.

Distribution: Prov. Minas Geraes, Brazil.

Rio Arassuahy, Glaziou A, fl. fr. (C, P); idem, Glaziou 13136, fl. June (C).

6. **Mourera alcicornis** (Tul.) v. Royen, nov. comb. — *Ligea alcicornis* Tul. (1852) 94—95; Walpers (1858) 780; Tul. (1863) 243, t. 73 f. 3 — *Oenone alcicornis* (Tul.) Wedd. (1873) 58; Warming (EP 1891) 18; Engler (1930) 37.

Small, usually stemless species. Leaves narrowly and sometimes obliquely cuneate, pinnatilobed, 1.5—9 cm long, 3—35 mm wide, with a few irregular lobes which are divided at the top into 3—6 mm long, acute threads, rough at the upper surface, palmatinerved. Flowers in more or less indistinct spiciform pedunculate monochasia or sessile; alternating with leaf-like bracts; monochasia branched or unbranched, up to 3 cm long; mature spathella narrowly infundibuliform, up to 2.5 cm long; pedicel 1.5—3.5 cm long; tepals 8—10, linear, acute, about 0.5 mm long; stamens 8—10, from 4 to 4.5 mm long; sometimes 2 or 3 filaments united at the base and then the vascular strands in this part free or united; anthers 1—2.5 mm long, obtuse or emarginate; base of the thecae obtuse or mucronate; pollen grains 18 μ high, 11 μ diam; ovary globose, substipitate, 1—2.5 mm long, 1—1.5 mm diam, with 6 ribs; styles filiform, free or cohering at the base, 1—1.5 mm long. Fruit similar to the ovary, up to 3 mm high.

Type: Spruce 555, P, duplicates B, CGE, G.-Del., GH, K, M, NY, W; collected in Brazil.

Distribution: Northern Brazil.

Rio Aripecuru, prov. Para, Spruce 555, fl. fr. Dec. (B, CGE, G.-Del., GH, K, M, NY, P, W); idem, Cachoeira do Tronco, Ducke 14994, fl. fr. Oct. (CGE); Rio Gatapy, prov. Amazonas, Barbosa s.n., fl. fr. Sept. (CGE).

DUBIOUS SPECIES.

1. **Mourera penicillata** Hicken (1917) 148.

Latin description of the species: "Rhizomate crasso lignoso 6 mm anguloso; foliis 10—30 cm longo, 3—10 cm lat., amplis runcinato-lobulatis, lobulis inaequalibus sinuatis in apicibus sinuum tantum longiuscule penicillatis; penicillis ad 10 cm longis. Laminae basi assymetrica; nerviis furcatis, haud anastomosantibus, extremus in penicillis desinentibus; pagina superiore papillis numerossisimis asperata. Petiolo breve 1—3 cm longo, ad basem foliae paullo expanso vel alato.

Obs. Specimina sterilia hac de causa genitalia invisa sed mihi ob habitum necnon penicilli certe novam speciem extat. Mourera aspera Bong. affinis, sed ambitu, lobulisque et conis laminae sat distincta."

Type: Rodriguez 793, Buenos Aires, collected in Argentina.

Distribution: Known from the type-locality only.

I have not been able to study the type-specimen, and as it is sterile, it is difficult to say whether it represents a distinct species. Judging from the description reproduced above, this seems highly doubtful; as was pointed out by Tobler (1933) for Mourera aspera, the margin of the leaf may in these plants be dissected in numerous lobes each ending in a few filiform segments. However, as this division is apparently due to the impact of the water, by which the leaf is partly destroyed, the remaining part reacting by the development of these segments, there is good reason to doubt the specific nature of the difference between this specimen and normal Mourera aspera.

4. **TULASNEANTHA** v. Royen, nom. nov.

Medium-sized, stemless herbs provided with a short base. Leaves distichous, provided with a distinct, smooth petiole; lamina flabelliform, repeatedly forked. Flowers in long 2-sided spiciform monochasia, springing from the axil of the upper leaves; flowers alternating with boat-shaped bracts; spathella clavate, acuminate, slightly exceeding the bract; petiole decurrent in the rachis over a long distance; tepals 6—10, in a complete whorl, very small; stamens 6—10, in a complete whorl; filaments united halfway or slightly less; anthers sagittate, dehiscing introrsely; pollen grains globose; ovary ellipsoidal, attenuate at the base, consisting of 2 equal cells and provided with 8 ribs; styles 2, filiform, cohering at the base. Fruit slightly protruding beyond the spathella, with 2 equal persistent valves.

Type: *Tulasneantha monadelpha* (Bongard) v. Royen.

Distribution: One species collected in Western Brazil. **(See map)**

In 1775 Aublet described *Mourera fluviatilis* as the first species of what later became the family *Podostemaceae*. For an unknown reason Schreber (1789) changed Aublet's generic name in *Lacis*, but no other species were referred by him to that new genus. In 1824 Martius and Zuccarini described some other species for which they used the generic name *Lacis*, and in 1835 Bongard too published some new *Lacis* species, i.a. *Lacis monadelpha*. In the same year von Chamisso described some new *Marathrum* species, and returned *Lacis fluviatilis* to *Mourera*, where it correctly belongs. However, as *Lacis fluviatilis* is the type of *Lacis*, this genus could not be maintained. This was pointed out by Lindley (1836) but the latter proposed to solve the difficulty by conserving the name *Lacis* for *Lacis monadelpha*, this species being the only one that could not be included in other genera. This, however, is contrary to the international rules of nomenclature, as the type

of *Lacis* is *Mourera fluviatilis*; moreover the whole description of *Lacis* had to be changed in order to adapt it for the reception of *Lacis monadelpha*. I therefore propose to name this genus *Tulasneantha*, in honour of TULASNE, the first monographer of the *Podostemaceae*. (See also v. Royen (1951) p. 9).

1. **Tulasneantha monadelpha** (Bongard) v. Royen, nov. comb. — *Lacis monadelpha* Bongard (1835) 78, t. 1 f. 1—6; Lindley (1836) 442; Endlicher (1836) 270; Tul. (1849) 94; Walpers (1852) 433; idem (1858) 776; Tul. (1863) 238—239; Schnitzlein (1847—'70) t. 585 f. 6; Weddell (1873) 51—52; Baillon (1888) 260; Warming (EP 1891) 20; Engler (1930) 44, f. 33 — *Lacis bongardii* Tul. (1852) 69—70.

Base branched or unbranched, terete, 1—5 cm long, 0.5—2 cm high. Leaves 10—30 cm long; petiole membranaceous, slightly widened at the base, 5—16 cm long, 1—3 mm wide; ultimate divisions of the blade with a distinct nerve. Inflorescences unbranched, compressed, 8—30 cm long; peduncle terete, up to 20 cm long, at the base with two distinct, about 1 mm wide wings, which taper towards the top. Flowers numerous, about 0.5 cm apart; pedicel 1—3.5 cm long, slightly widened at the top; juvenile spathella clavate, acuminate; mature one infundibuliform, 0.5—1 cm long; bracts lanceolate to elliptical, acuminate, slightly folded and crested, 0.5—1 cm long; tepals 6—10, about 0.5 mm long, lanceolate, sometimes absent; stamens 6—10, from 5—12.5 mm long; anthers narrow, obtuse; base deeply incised; the thecae obtuse or emarginate; pollen grains incompletely known; ovary narrowly obovoid, acute, 3.5—8 mm high, 1.5—2 mm diam; midrib slightly winged at the top; styles with rostriform top and 3-edged base, 1.5—4 mm long, cohering.

Type: Riedel 1268, herb LE—I, duplicates B, G.-Boiss., P, U; collected in Western Brazil.

Distribution: Western Brazil.

Rio Madeira, Riedel 1268, fl. fr. (B, FI, G.-Boiss., P, U); Para-district, Riedel s.n., fl. fr. (P); without loc., Riedel s.n., fl. fr. July (P); without loc., Ferreira 353, fl. fr. (K).

5. LONCHOSTEPHUS Tul.

Small, stemless herbs provided with a short base. Leaves radical and distichous, repeatedly forked; ultimate divisions filiform. Flowers in few-flowered, unbranched, 2-sided, spiciform monochasia springing from the axils of the leaves. Flowers alternating with boat-shaped bracts; bracts sometimes foliaceous; basal margin of the higher bracts covering the apical margin of the preceding ones; juvenile spathella clavate; mature one infundibuliform; tepals 5—8, in a complete whorl, free, filiform to subulate, very small; stamens 5—8, in a complete whorl, free; filaments widened, membranaceous, elliptic; anthers sagittate, dehiscing introrsely; pollen grains ellipsoidal, 3-

colpate, covered with many small warts; ovary ellipsoidal, attenuate at the base, with two equal carpels and provided with 8 ribs; styles cristate, free. Fruit with two equal, persisting valves; each valve with 3 ribs.

Type: *Lonchostephus elegans* Tul.

Distribution: One species in the Amazon-river, Brazil. **(See map)**

The single species, *Lonchostephus elegans*, was regarded by Tulasne as representing a new genus, but Baillon referred it to *Mourera*, as according to him the widened filaments and the cristate styles are mere variations of those described e.g. in *Mourera fluviatilis*, were the styles are filiform and the filaments narrow lanceolate. However, it seems to the present author that the cristate style justifies the recognition of a separate genus.

1. **Lonchostephus elegans** Tul. (1852) 198—201; Walpers (1858) 776—777; Tul. (1863) 239—240, t. 73 f. 2; Weddell (1873) 51; Warming (1891) 20; idem (1899) 118—120, f. 11—12; Engler (1930) 42—43, f. 32 — *Mourera elegans* (Tul.) Baillon (1888) 260.

Base short, irregular, about 1 cm long and 2—5 mm wide. Leaves 1.5—8 cm long; petiole cuneate, compressed, widened at the base and provided with two wings. Inflorescences 1.5—8 cm long; peduncle 4-edged or, sometimes, slightly winged, at the top with one or a few, lanceolate to linear, pinnatipartite, 0.5—3 cm long leaves with at the top finely dissected lobes. Flowers few; pedicel 0.5—2.5 cm long; juvenile spathella obtuse or mucronate; mature one 4—10 mm long; bracts boat-shaped, about 5 mm long, sometimes developed as a small leaf and provided with an intrapetiolar sheath, in other instances cristate and ending in an acute point; basal margin or the tip of the sheath only provided with several small teeth; tepals lanceolate, acute, 0.5 mm long; stamens 4—6.5 mm long; filaments 3—5 mm long, 1—1.5 mm wide; anthers obtuse; base of the thecae obtuse to truncate, pollen grains 19 μ high, 12 μ diam; ovary borne by a short gynophore, provided with 8 ribs, 3—6 mm long, 1—2 mm wide; styles free, 1 mm long or less. Fruit similar to the ovary.

Type: Spruce 631, P, duplicates CGE, K, M, S; collected in Brazil.

Distribution: Upper Amazon, Brazil.

Amazon-river, Spruce 631, fl. Aug. (CGE, K, M, P, S); Amazon-river near Santarem, Spruce 1036, fl. fr. Aug. (B, BM, C, F, FI, GH, G.-Boiss., G.-Del., GOTT., K, P, W).

LIST OF COLLECTORS NUMBERS

The collectors' numbers are printed in *italics*; the numbers in parentheses are the pages on which the collectors' numbers are cited. "*S.n.*" mean sunnumbered specimen.

Schwacke *3032* (7); *4986* (12); *5011* (7).
Skutch *2473* (7); *2522* (7); *4603* (7).
Smith, A. C., *2103* (9); *2139* (12); *2648* (12).
Spruce *s.n.* (7); *555* (15); *631* (18); *1036* (18); *1084* (7); *2752* (9).
Staal *s.n.* (7).
Standley *1123* (6); *1696* (6); *3990* (6); *8004* (6); *8936* (6); *9662* (6); *29878* (7); *29933* (7); *29934* (7); *49291* (7); *55915* (6); *56283* (6); *56284* (6); *60408* (6); *75497* (6); *78604* (6); *79471* (6); *79714* (6); *87867* (6); *88000* (6); *88002* (6); *88248* (6); *88311* (6); *88363* (6); *88817* (6).
Standley & Chacon *5604* (6); *6743* (6).
Standley & Molina *4660* (6).
Standley & Padilla *2595* (6); *2763* (6); *2784* (6); *3827* (6).
Standley & Valerio *44263* (7); *45031* (7); *45033* (7); *45684* (7); *46346* (6); *46615* (7); *46633* (7); *46656* (7).
Steyermark *32914* (6); *33363* (6); *33368* (6); *33576* (6); *34548* (6); *39119* (6); *42113* (6); *46314* (6).
Steyermark & Allen *17130* (7); *17257* (7); *17400* (7).
Sumichrast *s.n.* (6).
Tobler *s.n.* (14).
Tonduz *7126* (6); *9838* (7); *11294* (6).
Torres *3* (7).
Traill *802* (12).
Tresling *81* (12); *101* (12).
Tulleken *438* (11).
Tutin *27* (12).
Ule *7966* (12).
Valerio *1130* (7).
Vauth (-ier) *s.n.* (14).
Versteeg *46* (11); *150* (11); *610* (11); 749 (11).
Weber *s.n.* (6, 12).
Weddell *s.n.* (7); *2320* (13); *2366* (7).
Wendland *s.n.* (6, 7).
Went s.n. (12); *462* (11).
Williams *1043* (7).
Wright *s.n.* (6); *3195* (7).
Wullschlaegel *1673* (11).
Yuncker c.s. *8853* (6).

GENERAL INDEX

Synonyms are printed in *italics*; new taxa are printed in **bold** face type; previously published names in ordinary type.

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THE EFFECT OF INDOLE ACETIC ACID AND OTHER GROWTH PROMOTING SUBSTANCES ON THE ENDOGENOUS RESPIRATION OF THE AVENA COLEOPTILE

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CONTENTS

GENERAL INTRODUCTION

§ 1. Aerobic respiration as a "formal prerequisite" for growth

The first investigations, indicating that growth by cell elongation is connected with the aerobic respiration, date back to the last century.

Wiesner (1878) reported that "heliotropische Erscheinungen" (phototropic curvatures) in seedlings of *Phaseolus multiflorus*, *Vicia faba* and other plants only occurred when there was free oxygen in the environment.

Wortmann (1880) noticed the same with the roots of seedlings of these plants: without free oxygen growth and geotropic response failed to occur.

Analogous results were obtained by Correns (1892) with various seedlings and by Van Ameyden (1917) with *Avena* coleoptiles: in a nitrogen atmosphere neither phototropic nor geotropic curvatures took place.

Aerobic respiration was understood in those days as a "primary essential" (Pfeffer, 1900) for growth in the way that respiration was merely the source of energy for the growth processes, which appears, for instance, from Wortmann's statement (l.c. p. 520) of the problem. This author asked himself: "weshalb die intramoleculare Athmung allein für die Pflanzen keine Kraftquelle ist, weshalb nur durch das Eingreifen des atmosphärischen Sauerstoffes die Kräfte frei werden, welche das Wachstum bewirken" (why in plants the intramolecular respiration does not suffice as a source of energy, why only by the action of atmospheric oxygen the energy is set free, which causes growth).

A new attack on the problem of the relation between growth and respiration was made after the discovery of the growth substances. The early statement of Went (1928) that growth substances are in-

dispensable for growth has been more and more widely accepted and many investigations were started to detect the locus as well as the mode of action.

Originally, auxin action was localised in the cell wall (Heyn, 1931; Ruge, 1937, 1942). Then the protoplasmic membrane was considered as the probable locus of action (Koningsberger, 1942, 1947; Veldstra, 1944). Finally, the auxins were thought to be active inside the protoplasm. Recent literature does not leave much doubt, that growth substances cause physico-chemical and (or) chemical changes in nearly all parts of the cell. This does not include, however, that all these changes are supposed to be the result of a direct action on the spot.

In theories on growth substance action one often meets with the view that they do not act on different cell components separately but that their action is confined to control in some way a master system. The diverse phenomena resulting from the supply of a growth substance would have to be considered as secondary reactions which were started by, what Thimann (1935) called, "the master reaction". A distinction was therefore made between the primary effect of a growth substance, which was thought to act upon a hypothetical master system, and diverse secondary reactions.

Commoner and Thimann (1941) considered the primary action to be exercised on the respiration. Growth would be closely linked to that part of the respiratory process which is called the Szent Gyorgyi — Krebs- or C_4-dicarboxylic cycle. The authors showed that the growth of *Avena* coleoptile sections in sucrose and auxin solutions was inhibited by substances which are known to inhibit dehydrogenases. Particularly, iodoacetate appeared to be very active in this respect as, with a concentration of 5×10^{-5} M growth was stopped entirely, whereas the respiration was only reduced to about 10 %. The growth inhibition could be neutralized by addition of components of the above-called cycle (malate, fumarate and succinate) and also by pyruvate. Besides, addition of these acids increased the growth promoting influence of indole acetic acid (I.A.A.). Further, the respiration of sections, soaked in malate or in fumarate, was increased from 15–28 percent by I.A.A. It was concluded that the "four-carbon acids provide a respiratory system which is part of the chain of growth processes, and which is in some way catalized by auxins. It represents a small but variable fraction of the total respiration" (l.c. p. 295).

In this theory the respiration is *placed into* the chain of processes which result in growth. Instead of being only a "formal prerequisite", an energy providing process, it is held that substances which promote the growth act *via* the respiration.

The ideas of Commoner and Thimann have been further developed by Thimann and Bonner (1948, 1949, 1950) in a series of papers on the growth and inhibition of isolated plant parts (*Avena* coleoptile sections and *Pisum* internodes). Experiments with more specific sulfhydril inhibitors confirmed earlier investigations and led to the final

conclusion that growth is controlled by an enzyme (or co-enzyme) which contains a sulfhydril group, and that the organic acids of the Krebs-cycle represent the central link between the diverse metabolic processes which are involved in growth (see also Thimann, Bonner and Christiansen, 1951).

In a recent communication on the mechanism of auxin action Thimann (in Skoog, 1951) considered auxins as ideal protectors of the organic acid metabolism against natural inhibitors. According to his opinion auxin combines with the enzyme to be protected. The adsorption power to this enzyme must necessarily be stronger than that of the hypothetical inhibitor.

In spite of the criticism on the above-mentioned theory (see Berger and Avery, 1943, who showed that their results were open to other interpretations), the significance of the organic acid metabolism for growth has been recognized even by those authors who localized the auxin action in the phosphate metabolism (see later).

2. The influence of growth substances on the oxygen uptake

A special aspect of the problem of the relation between growth, growth substances and the aerobic respiration, which has been studied in many investigations, including the present one, is that of the ability of the growth substances to increase the oxygen uptake of plants and tissues. Most of these studies were concerned with the exogenous respiration, the substrates (sucrose) being supplied in excess. It is obvious, that, when the latter is the case, the rate of oxygen consumption is controlled by the capacity of the respiratory enzyme system. The stimulative action, found in these experiments (Commoner and Thimann (1941), Berger, Smith and Avery (1946), Kelly and Avery (1949), Anker (1951) et al.) was, therefore, undoubtedly exercised on the enzyme controlling the rate of the substrate oxydizing process.

In other investigations the above-mentioned problem was studied under entirely different conditions, namely of mild or strong starvation of the respiring tissues. Since in this case the substrate is limiting factor, it is evident that any stimulative effect under these conditions must be excercised on the substrate supply.

The present study belongs to the latter group of investigations, as the course of the respiration after I.A.A.-addition was studied with *curved Avena* coleoptile sections.

The information available from the literature shows, that no unanimity exists about the quantitative affectibility of the respiration when substrate is short. No stimulation was found by Commoner and Thimann (1941), Berger, Smith and Avery (1946), whereas, even considerable increases of the endogenous respiration were found by Bonner (1949) and Kelly and Avery (1949).

In the present investigation of the problem, with methods slightly different from those used by the authors mentioned-above, results are obtained which leave no doubt about a promoting influence of growth substances on the endogenous oxygen uptake.

A detailed study of the effect was made with sections cut from different positions on the coleoptile, after varying periods of starvation and in connection with the growth of the sections.

Next, the effect produced by other growth promoting substances was compared with that of I.A.A. and, finally, experiments were carried out concerning the influence of I.A.A. on starch hydrolysis by amylases *in vitro*.

For the explanation of the results, theories, based on the assumption that growth substances act on special enzymes or as components of enzymes, had to be discarded.

It is held, that the observed aspect of growth substance action is best explained by claiming a sensibilizing effect on inner lipophilic protoplasmic films, which, presumably, separate the enzymes and their endogenous substrates.

This view is a modification of the ideas of KONINGSBERGER (1942, 1947) and VELDSTRA (1944) who regarded only the external protoplasmic membrane as a lipophilic film.

CHAPTER I

MATERIAL AND METHODS

Seeds of *Avena sativa* (Victory Oat, Svalöv, 1949), after having been soaked in tap water for three hours, were sown on moist saw dust and grown in a dark room at 23° C and 90–96 % relative humidity. After 4 to 5 days the coleoptiles had reached the length required for manipulations. The coleoptiles were severed from the mesocotyl and about 5 mm were removed of the *lower* part, so that the primary leaves could be pulled out by hand. Next, the "empty" coleoptiles were divided into 3 mm sections with a cylinder microtome. The tips of the coleoptiles (4 mm) were rejected. Of each coleoptile three sections were cut. The sections of a given position were kept apart. Thus the influence of I.A.A. could be studied separately on the top sections (5–7 mm of the original coleoptile), the middle sections (8–10 mm) and the bottom sections (11–13 mm).

These sections were either used directly or after a 24 hours period of starvation. During starvation, they floated on buffer (KH_2PO_4, pH = 4.5) solutions after having been mounted on thin, massive glass rods. It appeared that by complete submersion of the sections the capacity of the respiratory enzyme system was reduced. Finally, the starved sections could be easily slipped into the respiratory vessels. Each vessel contained 30 sections.

The gas exchange was measured in Warburg respirometers. The speed of the rotating shaker was about 240 oscillations per minute. The sections were suspended in KH_2PO_4-solutions, the concentration of which will be given with the experiments. For CO_2-absorption, the central well contained 0.25 ml of a 10 %KOH-solution. By addition of filter paper the absorbing surface was enlarged.

Before adding the growth substance to the experimental vessels, the rates of oxygen uptake in control and experimental vessels were compared for two hours. In the ideal case these rates are equal. The actual differences, however, due to the natural variability of the material, were not negligible as is shown by the last column of Table I.

TABLE I

Comparison of the oxygen uptake in three vessels (a, b and c) containing 30 sections from the same position of the original coleoptile (5–7 mm).

Experiment	mm^3 O_2/hour			maximal difference in % of the lowest value
	a	b	c	
1	21.1	22.0	20.5	7
2	25.2	26.2	30.3	20
3	17.6	18.7	17.8	6
4	23.1	22.9	23.5	3
5	20.1	21.7	19.8	10
6	19.7	20.4	19.0	7
7	26.0	25.8	24.9	4
8	17.1	18.6	17.3	9
9	22.7	22.2	21.3	7
10	21.3	22.6	22.4	5

These differences in the initial rate of O_2-uptake were taken into account when the percentages stimulation or inhibition by a given growth substance were computed.

In order to be able to add the growth substances, the vessels were detached from the manometers. Care was taken that no liquid from the thermostat could enter the vessels (wiping with cotton wool). 0.5 ml of a growth substance solution (concentration five times that of the desired final concentration) was pipetted in the vessel making the total volume of the medium 2.5 ml. To the control vessels the same amount of destilled water was added.

This way of adding was preferred to tipping the growth substance in from the side vessel. For, quantitative transfer requires that the side-arms are washed with some fluid from the main compartment. In doing so, however, the sections get stuck at the walls of the main vessel or of the side-arm and it takes a long time of manipulation, before they are all back in the liquid, which is important for obtaining uniform conditions.

The growth substance solutions were prepared with distilled hot water and not by means of the addition of some ethanol. In the present experiments on the respiration of starved tissue it was not allowed, if ever, to use ethanol because this substance stimulates the respiration, as will be seen later.

During the whole experiment no white light was admitted to the sections. The readings were done in phototropically inactive orange light at 25° C.

No special care has been taken to avoid bacterial contamination. Only once bacteria were observed under the microscope at the close

of these long experiments. Here the oxygen uptake started to increase considerably after 7 hours and reached, within 10 hours, a level which was 3 times that of the normal amount of oxygen uptake per hour. The medium became turbid and many rod-shaped bacteria showed up under the microscope. The increase in oxygen uptake, when bacteria were present, was not followed by a decline, which appeared in cases where no contamination was found (see p. 29).

CHAPTER II

THE RESPIRATION OF THE AVENA COLEOPTILE

§ 1. Introduction

A considerable amount of research has been done on the respiration of the *Avena* coleoptile (see Bonner, 1948) and it has become clear that the biochemical mechanism is not different from that of other plant tissues. Measurements of the respiratory quotient of freshly cut coleoptile sections showed a value of approximately one, which is indicative for carbohydrate consumption.

Relatively few data are available concerning the *rate* of the respiration of this tissue, and in particular by which factors the rate is controlled.

This question was first studied by Bottelier (1939). When adding 2.5 and 5 percent of glucose to freshly cut sections, this author observed a considerable increase in the respiration, which led him to the conclusion that, normally, the substrates are the limiting factor in the process.

Bonner (1948) too, found that addition of 1 percent of sucrose caused 22 percent increase in the oxygen uptake of freshly cut sections. In later experiments (Bonner, 1949) the same sucrose concentration caused no increase in respiration. Here acids of the Krebs-cycle caused an appreciable response, which did not occur in the former experiments.

These results, when taken together, indicate that in the coleoptile sections the substrate concentration is or soon becomes the limiting factor in the respiration.

Since the interpretation of auxin effects on the respiration of coleoptile sections, suspended in buffer without substrate, made it necessary to settle this point, the experiments on this subject were repeated.

Next, the course of the respiration of coleoptile sections was studied during a period of 24 hours, also, when suspended in a substrate-free medium. These experiments were also preliminary to the study of the effect of growth substances on the respiration after varying periods of starvation.

§ 2. Experiments concerning the course of the endogenous respiration

Five-days-old coleoptiles were divided in 3 mm sections which represented the 5–7 mm, the 8–10 mm and the 11–13 mm of the

original coleoptile. The respiration of sections of a given position was studied separately. The experiments lasted from 4 p.m. to 4 p.m. or later on the next day. Interim opening of the vessels did not influence the respiration so that the oxygen pressure apparently remained above the critical value. In fig. 1 a few examples are given, taken from three different experiments.

The initial decline in respiration in the first hours is in agreement with the results of BOTTELIER and of BONNER. Rapid, as well as slow, declines were found. However, when the experiments were continued over a longer period, it appeared that, after about 12 hours, the gradual reduction in the respiration was followed by an increase in

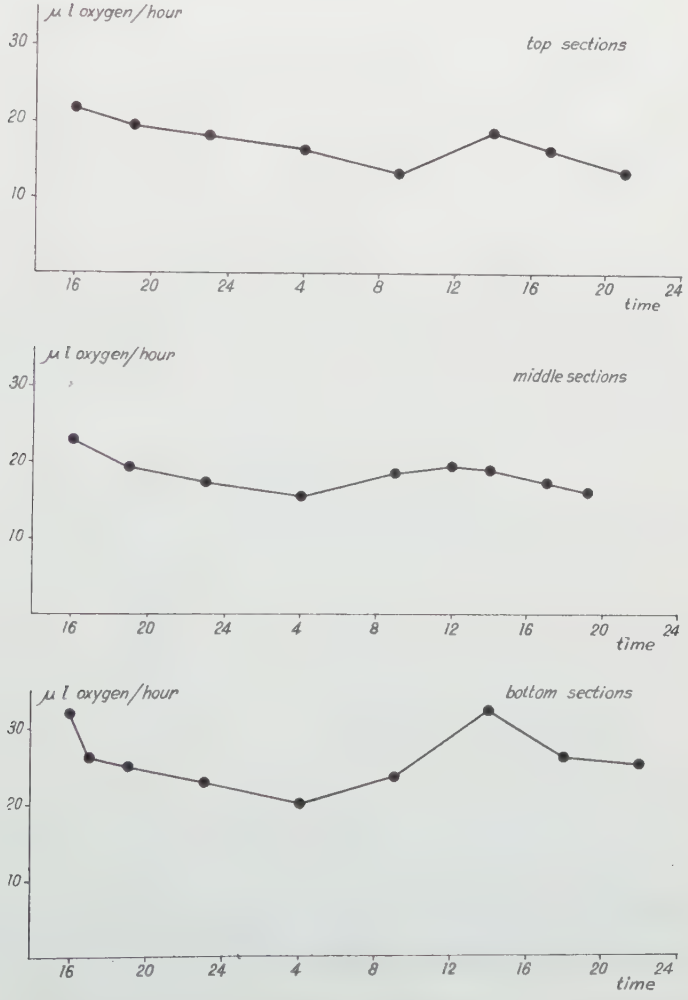

Fig. 1. The course of the endogenous respiration in *Avena* coleoptile sections, suspended in a substrate-free buffer solution. (three experiments)

the oxygen uptake, which, after another 10–12 hours, was followed by a new decline. This periodicity in the rate of the respiration was observable in all eight experiments, though, in some cases, it was not so evident as in the examples of fig. 1.

As a rule, the maxima on the second day reached about the level of the initial respiration rate. In this respect the bottom sections behaved differently, as the maximum on the second day surmounted the initial value. In fig. 4, p. 41, the courses of the endogenous respiration of top-, middle- and bottom sections of one experiment are comparable. Here too, the bottom sections reached a higher level on the second day. The curves of the oxygen values of sections of different levels of the coleoptile were not principally different and, generally, they ran parallel.

§ 3. Addition of glucose to starved coleoptile sections

It has been shown in the previous section that the intensity of the endogenous respiration varies periodically. These variations may, theoretically, be due to (1) variations in the activity of the oxydizing enzymes or to (2) variations in the intensity of the substrate mobilisation. The experiments of this section resulted in favour of the second possibility.

Sections of five-days-old coleoptiles were starved for 24 hours in the way described on p. 26. After the sections had been introduced into the 2 % KH_2PO_4-buffer, the rate of oxygen uptake was measured for 3 hours. Next, glucose was added to the final concentration of 0.5 %, after which the rate of oxygen uptake in the exogenous respiration was measured for another 3 hours. The quotients $\frac{\text{exogenous}}{\text{endogenous}}$ respiration of 7 experiments are summarized in Table II.

TABLE II

Quotients of $\frac{\text{exogenous}}{\text{endogenous}}$ respiration

Exp.	top sections	middle sections	bottom sections
1	1.95	2.43	2.43
2	2.08	1.81	1.98
3	1.65	2.17	2.36
4	2.10	2.04	2.08
5	1.88	2.13	1.88
6	1.40	1.64	1.64
7	1.81	2.02	1.83
Average	1.84	2.04	2.03

It appears from this Table that after adding glucose the respiration of the sections of all positions was about doubled, though the actual quotients varied from 2.43 (exp. 1) to 1.40 (exp. 6).

In these experiments the respiration was not measured during the preceding 24 hours period of starvation. It is therefore not known to what extent this process had been changed as compared to the respi-

ration of freshly cut sections. Still an estimation is possible, since the rates of oxygen uptake before adding glucose were of the same order as those mentioned in the experiments in the previous section. Here, after 24 hours, the average respiration had decreased about 10 % as compared with the initial value. Because of the fluctuations this average has to be accepted with proper reserve as appears from fig. 1c, where instead of a reduction an increase was observable after 24 hours. The conclusion is allowed, however, that the addition of glucose raised the respiration to a level which was much higher than that of freshly cut sections. This means, that (1) the respiratory enzymes were not much — if at all — harmed by the starvation and (2) that the respiratory rate of sections suspended in buffer is limited by the concentration of available substrate.

§ 4. Discussion

The experiments described above are an indirect confirmation of Bottelier's work, and therefore, they support the view that in starved as well as in normal coleoptiles the respiratory enzymes are not saturated with substrate.

The rate of the endogenous respiration appears to be about half of that of the exogenous one.

Ultimately the reserve polysaccharides are the substrate for the endogenous respiration, which, according to our own estimations, were found to be present as starch in large amounts, even after long periods of starvation.

The special suitability of starch to be used in the respiration has been emphasized by James (1946) in a review on the respiration of plants. The small difference in energy content between the glucose – glucose linkage and that of the phosphate-glucose linkage is the reason, that far less energy is needed to form the Cori-ester from starch than to transform free hexoses into phosphate esters.

Still more evidence is produced by R.Q. measurements on the basis of which Bonner (1948) presumed that the normal endogenous substrate is of a carbohydrate nature.

The fluctuations found in the intensity of the endogenous respiration remind one of the work of Bünning (1939). This author observed in photoperiodically active plants an endogenous rhythm in the activity of some processes. In the so-called night-phase many plants showed increased activity of the starch-hydrolyzing enzymes, which was accompanied by formation of free sugars and by an increased oxygen uptake. The rhythm in the oxygen uptake by the coleoptile of *Avena sativa*, which is a long day plant, might be of a similar character.

The maxima, however, were generally found in the early afternoon and not during the night. The cause of this divergence might be the differing external conditions. In the present experiments the seedlings were grown under constant conditions (darkness, constant temperature and humidity). The endogenous rhythm was, therefore, not affected

by the diurnal fluctuations of these factors, which presumably rule the "endogenous" rhythm under more normal conditions.

In the light of BÜNNING's work, the present fluctuations of the oxygen uptake become a strong indication that the rate of carbohydrate mobilisation determines the rate of the respiration in starved *Avena* coleoptile sections.

CHAPTER III

STIMULATION OF THE ENDOGENOUS RESPIRATION BY INDOLE ACETIC ACID AFTER A LONG PERIOD OF STARVATION

§ 1. Introduction

The first to study the effect of growth substances on the respiration of *Avena* coleoptile sections was J. BONNER in 1933. When adding a purified extract from the fungus *Rhizopus suinus* in different concentrations to sections which were cut 7 hours before, he observed an increase of about 20 %. This degree of stimulation was maintained for at least 4 hours. Thereupon a decrease to about the level of the control was observed, which took place within one or two hours.

In a second paper on this subject BONNER (1936) ascribed the above-mentioned effect to associated impurities in the extract of the fungus since he found no effect of pure auxins on the oxygen uptake in his new experiments.

In a third paper, BONNER (1949) returned to his original opinion as this time I.A.A. was found to enhance the oxygen uptake of *Avena* coleoptile sections both in the presence and in the absence of sugars in the medium. He states, however, not to have succeeded in finding an explanation for these varying results.

As for the influence of I.A.A. on the *endogenous* respiration of the sections, the next two papers confirm the negative results of BONNER's second paper.

The first came from COMMONER and THIMANN (1941), who obtained an increase in the respiration with various concentrations of I.A.A. only when sugar was present in the medium; with sections, respiring in water, no significant change in the O_2-uptake was brought about by I.A.A.

The same has been reported by BERGER, SMITH and AVERY (1946). I.A.A., added to sections in water, generally did not increase the respiration.

The main part of the present work is dedicated to this problem, which may be formulated as follows: do growth substances of the auxin type stimulate the aerobic respiration of *Avena* coleoptile sections when the substrate is the limiting factor in the process?

For this purpose sections were kept in a substrate free medium during 24 hours in the way described on p. 26. It was assumed that

after this period the respiration had become entirely dependent on the reserve carbohydrates and that the residual growth substance was about exhausted.

§ 2. Addition of I.A.A. to starved coleoptile sections

The experiments of this section have already been published in a preliminary note (Anker, 1951).

Sections from different levels on the coleoptile purposely were studied separately, whereas the cited authors mixed them. The I.A.A. was added after 24 hours to the final concentration of 1 mg/l. The results are summarized in the Tables III, IV and V and in Fig. 2.

a. The variability of the respiration

Before dealing with the influence of I.A.A. on the O_2-uptake, the attention may be drawn to Table III, where the respiratory rates of the controls of all (12) experiments are put together. This Table, when read in horizontal direction gives an impression of the wide variations of the respiratory intensity from day to day.

A very low rate was observed in exp. 1. This was, however, caused by the complete submersion of the sections during the preceding period of starvation. The lower rates in the experiments 10 and 11 are, presumably, due to the low buffer concentration (0.01 % KH_2PO_4) used. For the rest the influence of the buffer concentration on the intensity of the respiration, could not be made clear by these experiments because of the wide variations in the oxygen uptake in experiments at the same buffer concentration.

No influence of age and length of the coleoptiles, of which the sections were cut, on the respiration could be established with certainty.

Another type of variability is met with when Table III is read in vertical direction. As a rule, the rates of respiration in the top sections were exceeded by those of sections from lower positions on the coleoptile, the middle sections being intermediary. Deviations from this rule were found in exp. 6 (middle sections) and expts. 8 and 9 (bottom sections). The degree of increase in the respiration of the coleoptile in basal direction is shown in Table III by the quotient:

$$\frac{\text{respiration of bottom sections}}{\text{respiration of top sections}}.$$

This quotient, varying too, could not be correlated with length and age, nor with the respiration rates of the coleoptiles.

b. The variability of the effect of I.A.A. on the respiration

The effect of 1 mg/l I.A.A. on the respiration of starved sections of 3 different positions on the coleoptile was studied in 12 experiments. In 28 out of 36 (12×3) cases a stimulation was found, in one case no effect, whereas in 7 cases a slight inhibition of the O_2-uptake to a maximum of 6 % occurred. There was a great variability in the degree of stimulation (Table IV). In 20 out of 28 cases the percentage

TABLE III

The endogenous respiration of sections of different position on the coleoptile

No.	1	2	3	4	5	6	7	8	9	10	11	12
age [1]	4	4	4	4	4	5	4	5	4	4	5	4
length [2]	—	—	s	m	l	l	l	l	s	m	s	l
buffer [3]	2.5	2.5	2.5	2.5	2.0	2.5	2.0	2.0	2.0	0.01	0.01	1.0
top [4]	5.0 [7]	21.5	27.0	21.8	17.6	24.2	17.1	19.0	15.8	12.6	10.6	17.2
middle [5]	6.3	25.0	37.5	27.7	23.6	22.3	18.9	14.4	20.5	14.9	10.8	17.7
bottom [6]	8.3	28.4	48.8	36.0	30.0	25.6	22.6	14.0	20.2	19.2	13.3	19.1
bottom / top	1.7	1.3	1.8	1.6	1.7	1.1	1.3	0.7	1.3	1.5	1.3	1.1

[1] age of the coleoptile when sectioned, in days; [2] length of the coleoptiles: s = short = ca. 2 cm, m = medium = ca. 3 cm, l = long = ca. 4 cm; [3] concentration in % KH_2PO_4; [4] top sections = 5–7th mm of the coleoptile; [5] middle sections = 8–10th mm of the coleoptile; [6] bottom sections = 11–13th mm of the coleoptile; [7] respiration in μl oxygen per hour.

TABLE IV

The percent increase of the endogenous respiration after addition of 1 mg I.A.A. per liter

No. / duration in hours	1 / 6	2 / 3.5	3 / 4.5	4 / 5.5	5 / 6	6 / 4.5	7 / 2	8 / 4.5	9 / 3.5	10 / 3	11 / 3	12 / 6	average stimulation
top	+ 12	— 3	+ 44	+ 5	+ 15	— 2	+ 12	+ 17	+ 7	+ 15	+ 30	+ 12	+ 14
middle	+ 30	— 6	+ 18	0	+ 13	+ 17	+ 3	— 3	+ 3	+ 24	+ 45	+ 21	+ 14
bottom	+ 8	+ 9	+ 11	— 5	— 6	+ 28	— 4	+ 15	+ 8	+ 17	+ 17	+ 1	+ 8

TABLE V

The effect of I.A.A. on the endogenous respiration and its distribution in the coleoptile.

	stimulation				No effect	inhibition max. 6 %
	30 %	20–30 %	10–20 %	0–10 %		
top	1	1	6	2	–	2
middle . . .	1	3	3	2	1	2
bottom . . .	0	1	4	4	–	3
total. . . .	2	5	13	8	1	7

exceeded 10 %; in 7 cases an increase of over 20 % of the respiration was found (Table V). In some experiments the response to I.A.A. was low (Table IV, exp. 2, 4, 9), whereas in others the percentage of stimulation in all sections was high (exp. 3, 10 and 11). In a number of other experiments fairly high stimulations in sections of one level were accompanied by inhibition of the oxygen uptake in sections of another level (exp. 5 and 8).

From these results it is not possible to derive a conclusive answer to the question, in which zone of the coleoptile the respiration is most sensitive to I.A.A. It seems to be located in the region of the top and middle sections, though in three cases it is found in the bottom sections. This variation in the location of the maximal response cannot be correlated with age, length and intensity of respiration or growth of the coleoptile (for the growth rate see p. 51).

Nothing can be said here about the duration of the effect of I.A.A. on the endogenous respiration since, even at the end of the longest experiments an apparent difference in the respiration rates is observable. The percent effect is generally constant during the whole observation period, though a slight decrease at the end of the experiment may occur.

In Fig. 2 the course of the respiration in experimental and control

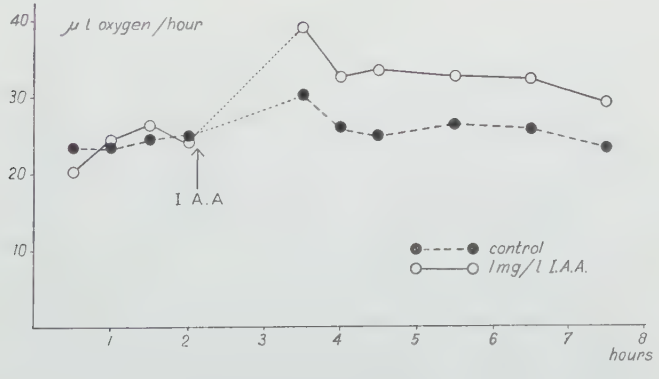

Fig. 2. The effect of I.A.A. on the rate of the endogenous respiration (experiment 6, bottom sections).

vessels is shown (exp. 6, bottom sections). Before I.A.A.-addition the amounts of oxygen taken up per hour were about equal; after the addition the respiration of the treated sections increased up to a level which was continuously about 25 % higher than that of the untreated ones.

In the next chapter the duration and other details of the effect will be described.

§ 3. Discussion

When searching for an explanation of the quantitative differences in the oxygen consumption from day to day, the differences in the size and shape of the coleoptile are, possibly, of primary importance. By factors, not yet under control, the coleoptiles vary from slender on one day to robust on another. Consequently, differing amounts of tissue are present in the respiratory vessels, which may largely account for the above variations. Aeration during the preceding starvation period and the concentration of the buffer solution are other factors determining the rate of the oxygen uptake.

Also the increase in the respiration in the basal direction of the coleoptile will be largely due to the fact that these organs end conically, the bottom sections being wider than those nearer to the tip. Parenthetically it is remarked that the zone of maximal growth certainly does not show the highest rate of respiration. The differences in diameter of top and bottom sections as well as the degree in variability of the diameters of the coleoptiles are shown in Table VI.

TABLE VI

Comparison of the diameters of top and bottom sections

Exp.		1	2	3	4	5	6	7	8
top sections	a	77	77	77	77	76	75	75	72
	b	70	70	70	70	65	62	62	62
bottomsections	a	88	88	88	86	86	85	84	84
	b	79	78	78	76	76	75	75	73

a = mean diameter in scale units in the direction through the vascular bundles ; b $\perp$ a

Observations of J. Bonner (1936) on the distribution of the respiration over the length of the coleoptile are in favour of the view that in the present experiments the oxygen uptake is determined by the quantity of tissue since in his experiments the respiration, calculated per mg coleoptile, did not change significantly from the 5th to the 13th mm of the coleoptile.

Besides the morphological differences, physiological factors may have influenced the ratio of the oxygen uptake in top and bottom sections. The respiratory activity per mg protein, which, according to J. Bonner (1936) decreases in basal direction may be one of these. Taken by itself, this decrease is an indirect indication of increasing substrate shortage in the basal direction, since the enzymes are unsaturated. Microscopical estimation of the starch content on different

levels of the coleoptile by the present author, though pointing in this direction, is considered insufficiently exact to support this supposition. Differences in the activity or the amount of starch mobilizing enzymes may, theoretically, also play a part in determining the intensity of the respiration at different levels.

The rate of the respiration and of the I.A.A.-increased respiration is not connected with the growth of the sections. This will be shown in Chapter VI, see p.51.

There is much variability in the reaction of the respiration to addition of I.A.A., (1) in the quantity and (2) in the localisation of the effect.

Generally the endogenous respiration is stimulated by I.A.A., such at variance with the conclusion which COMMONER and THIMANN and BERGER, SMITH and AVERY have drawn from their experiments. This controversy may partly be explained by differences in the method. The sections taken by these authors from different levels on the coleoptile were thoroughly mixed, while in the present experiments sections from a certain position were studied separately. Application of their method would also have resulted in none, or an insignificant increase in the expts 2, 4 and 7. The great increase found in other experiments (3, 10 and 11), however, are unaccountable by this methodological difference.

The small effect in the experiments 2, 4, 7 and 9 is possibly due to the fact that in some cases the reactivity of the cells to I.A.A. was about annihilated by the relatively long starvation period. In experiments reported in the next Chapter the starvation period was reduced to a few hours. Without any exception, a considerable increase in the oxygen uptake was found with this material.

These results, therefore, confirm BONNER's positive results.

With 2,4-dichlorophenoxy-acetic-acid (2,4-D), a chemical with auxin activity, similar results were obtained by SMITH (1948). After treatment of bean seedlings with this substance a strong stimulation of the oxygen uptake (2.5 to 4 times the corresponding rates in the untreated slices) was observed on the subsequent days in the slices of the first internodal tissue.

KELLY and AVERY (1949), using *Avena* coleoptile sections, after a starvation period of 18 hours in distilled water, by which the respiration was reduced to about two third of the initial one, obtained stimulation of the oxygen uptake after addition of 2,4-D. The extent of the increase was dependent on the concentration used. With 1 mg/l stimulation of 10 to 20 % was found, which equals that in the present experiments with 1 mg/l I.A.A. Their results with *Pisum* in principle correspond with those reported for *Avena*.

These data from the literature, together with our own observations justify the conclusion that stimulation of the endogenous respiration is a real aspect of growth substance action.

Besides the variations in the quantity of the effect, differences are

found in the locus of maximal effect. The conclusion that this is generally within the first 10 mm of the coleoptile seems permissable. No correlation can be found between the place of maximal effect and such factors as age, length, respiration etc. of the coleoptile. To be able to determine whether these variations are due to a shift of the zone of maximal sensitivity to auxin, experiments with various concentrations of I.A.A. are needed.

The great variability of the *Avena* coleoptile in its reaction to added growth substances is not limited to the respiratory response. The growth response too was found to vary in such a way that results obtained on different occasions were hardly comparable (THIMANN and SCHNEIDER, 1938; SCHNEIDER, 1938 and BENTLEY, 1950). The last named authors stress the necessity of avoiding comparison of growth reactions of sections taken from different positions on the coleoptile.

CHAPTER IV

STIMULATION OF THE ENDOGENOUS RESPIRATION BY INDOLE ACETIC ACID AFTER A SHORT PERIOD OF STARVATION

§ 1. INTRODUCTION

In this Chapter experiments concerning the effect described in the previous one are put together. They were carried out with freshly cut sections, the I.A.A. being added within three hours after sectioning (during the first hours after sectioning the relative intensities of the oxygen uptake of the sections in experimental and control vessels were determined; see p.27). The main purpose was to determine the duration of the effect and, since for this reason the respiration had to be followed over a much longer period, it was deemed better to use fresh sections in order to avoid complications by possible degenerations of the tissues, as well as to reduce the risk of contamination. Attention was paid not only to the duration of the effect but also to the first and to the last hours of its appearance. Finally, the possibility was investigated, whether the limited duration of the effect was due to depletion or inactivation of the I.A.A. or to other factors.

§ 2. THE INITIAL EFFECT

Even in experiments in which I.A.A. strongly stimulated the respiration (when the total oxygen consumption in treated and untreated sections was compared) the effect was often negative during the first hour. The initial course of the respiration, however, differed according to the position of the sections on the coleoptile.

Of ten experiments, in which the effect was on the whole greatly promoting in all sections, the type of the effect during the first hours is given in Table VII. It appears that the stimulative action of I.A.A.

starts sooner in the middle sections than in the top and bottom sections and is mostly not preceded by a period of inhibition.

TABLE VII

The initial effect of I.A.A. on the endogenous respiration of sections from different position on the coleoptile (10 experiments)

time after addition	top sections		middle sections		bottom sections	
	+	—	+	—	+	—
60 minutes	3	7	9	1	2	8
90 minutes	6	4	9	1	7	3
120 minutes	10	0	10	0	9	1

+ = stimulation
— = no effect or inhibition

§ 3. The level of the I.A.A.-increased respiration

As a rule — it has been shown earlier — the respiration of freshly cut sections, being put in buffer solutions without substrates, declines

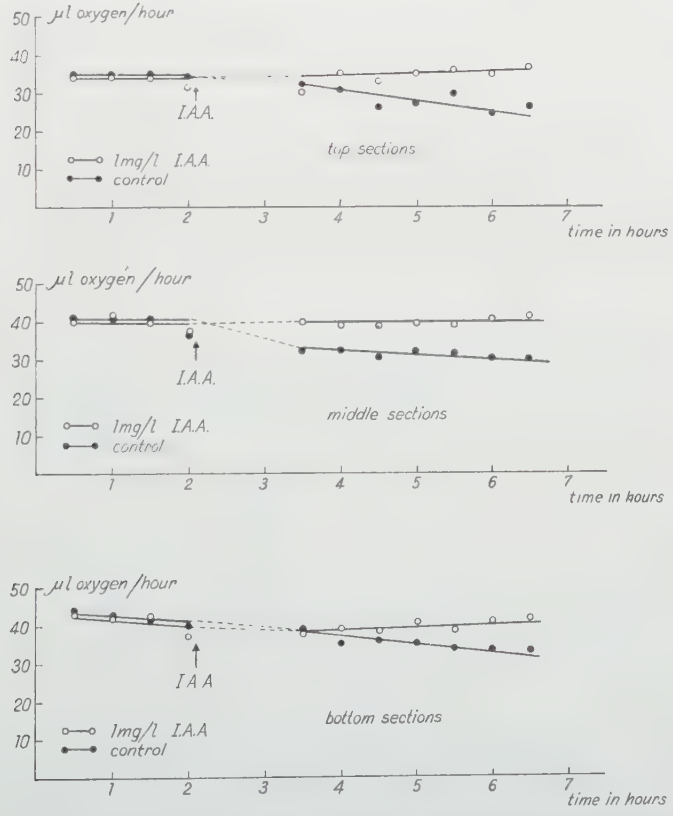

Fig. 3. The rate of the endogenous respiration is maintained on the level of that of freshly-cut sections by addition of I.A.A.

gradually with time. This decline starts either immediately or only after one or two hours. In one experiment sections of different position behaved differently in this respect. This is illustrated by Fig. 3, where the top and middle sections maintained a constant level of oxygen uptake during the first hours, whereas in the bottom sections the respiration declined from the very beginning.

This figure further shows the fact, already mentioned in the previous section, that the stimulation by I.A.A. was first observed in the middle sections.

The main reason, however, why this experiment is mentioned is to illustrate that the initial level of the respiration may be maintained for many hours when I.A.A. has been added. The effect of I.A.A. may therefore be described in this and other cases as delaying the decline of the respiration.

A similar observation was made by MICHEL (1951). In his experiments the oxygen uptake of hypocotyl sections of the Kidney bean declined rapidly in the controls, whereas the original value was maintained when I.A.A. was added previously.

§ 4. THE DURATION OF THE EFFECT

In nine experiments the respiration of untreated and I.A.A.-treated sections was followed over a period of 30 hours in order to investigate the duration of the stimulation.

This turned out to be as variable as are the quantity and the localisation of it. The average effect lasts nearly 20 hours in all sections. The results of six experiments with a 1 mg/l concentration and of three with other concentrations of I.A.A. are shown in Table VIII. It is clear from the detailed data (see below) that no much theoretical value should be attached to the averages.

TABLE VIII

Duration of the effect in hours

Exp.	concentration	top sections	middle sections	bottom sections
1	0.25 mg/l I.A.A.	21	14	14
2	1 mg/l I.A.A.	16	15	9
3	1 mg/l I.A.A.	3	25	25
4	0.50 mg/l I.A.A.	20	24	22
5	2 mg/l I.A.A.	21	16	19
6	1 mg/l I.A.A.	23	13	14
7	1 mg/l I.A.A.	30	24	22
8	1 mg/l I.A.A.	23	22	29
9	1 mg/l I.A.A.	17	12	17
	Average	19	18	19

Variations were also observed in one and the same experiment. ong-lasting effects in the top sections were accompanied by short timulations in the other sections and reversely.

One of the causes ending the effect was a rise in the respiration of the untreated sections to the level of that of the treated ones. This is illustrated in Fig. 4. When comparing in this Figure the respiration rates in top, middle and bottom sections of the experimental vessels,

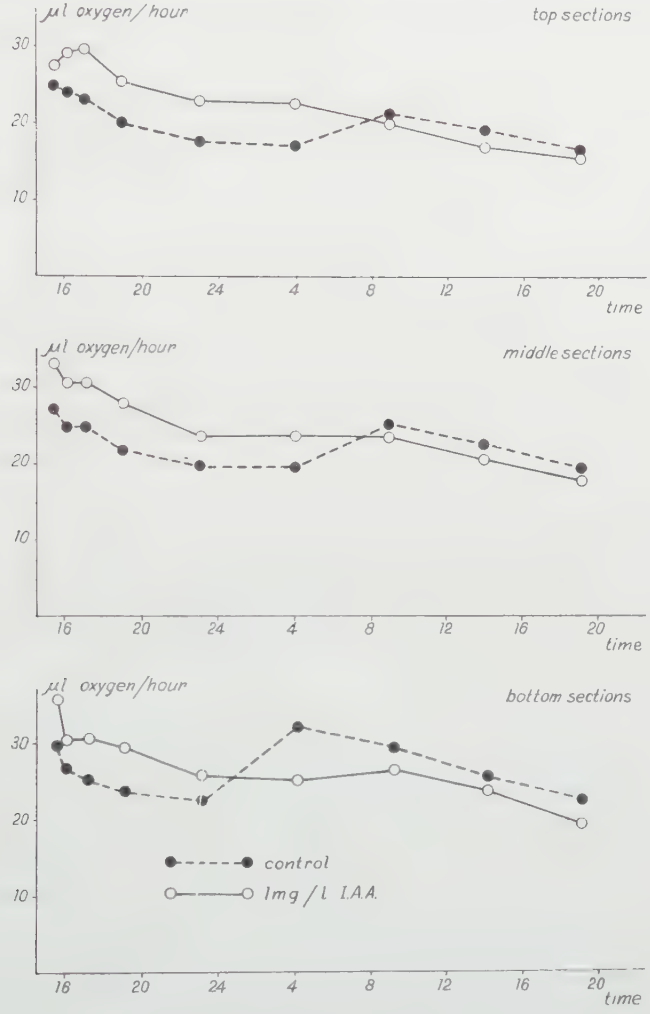

Fig. 4. The stimulation of the endogenous respiration caused by a 1 mg/l I.A.A.-concentration.

one observes that these do not fluctuate and run about parallel. As this is not so with the respiration in the control vessel, the effect is considerably curtailed in the bottom sections, since the increase in the oxygen uptake in these sections occurs much sooner than in the top and middle sections.

Further it is notable that the phenomenon, described in the second Chapter (p. 28) as an endogenous rhythm in the intensity of the respiration only occurs in the untreated sections.

In other experiments the effect ends more "normally" by a gradual decrease in the respiration of the I.A.A.-treated sections to the level of that in the untreated ones. This is shown in Figure 5, where ex-

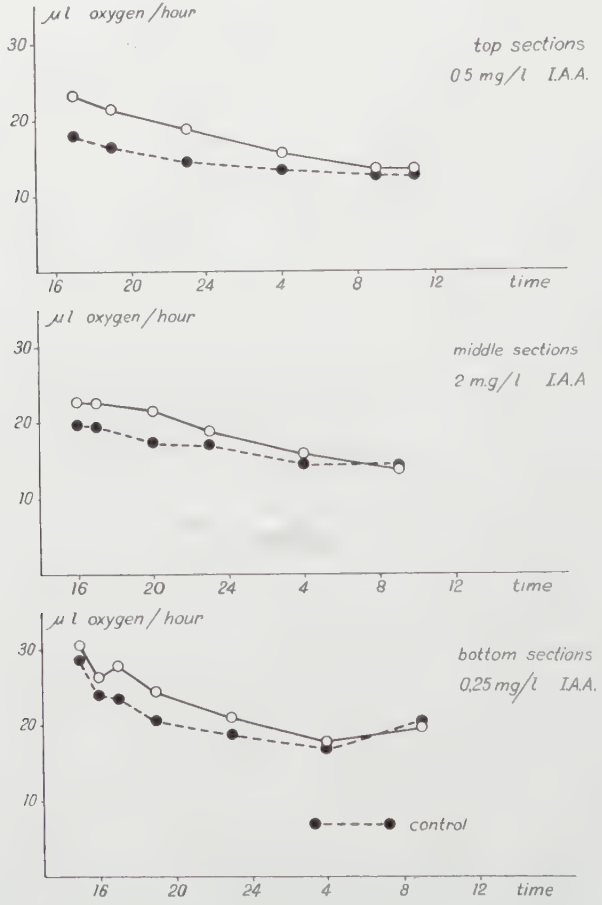

Fig. 5. The stimulation of the endogenous respiration caused by I.A.A. in the concentrations of 0.25, 0.5 and 2 mg/l.

amples, selected from three experiments with different I.A.A. concentrations (0.25, 0.5 and 2.5 mg/l) are given.

§ 5. The quantity of the effect

In the previous Chapter experiments were described concerning the influence of I.A.A. on the respiration of sections after a 24 hours' period of starvation. The results varied much in a quantitative sense.

Even in some cases no or a negative effect was observed. Much smaller variations were observed in the present experiments where I.A.A. was added within three hours after sectioning (see Table IX).

TABLE IX

Percentage of stimulation of the endogenous respiration of *Avena* coleoptile sections during the first 12 hours of observation.

Exp.	concentration	top sections	middle sections	bottom sections
1	1 mg/l I.A.A.	+ 33	+ 24	+ 5
2	1 mg/l I.A.A.	— 4 [1]	+ 11	+ 28
3	0.25 mg/l I.A.A.	+ 15	+ 13	+ 12
4	2 mg/l I.A.A.	+ 33	+ 12	+ 14
5	0.50 mg/l I.A.A.	+ 26	+ 23	+ 17
6	1 mg/l I.A.A.	+ 33	+ 22	+ 30
7	1 mg/l I.A.A.	+ 29	+ 27	+ 25
8	1 mg/l I.A.A.	+ 25	+ 11	+ 26
9	1 mg/l I.A.A.	+ 38	+ 42	+ 34
	Average	+ 25	+ 21	+ 21

[1] During the first hours the effect was strongly positive; by an uncommonly large increase in the respiration of the control sections, however, the effect became on the whole slightly negative.

Whereas it was concluded in the previous Chapter that stimulation occurred "generally", in these experiments the effect was always present. The average increase during the first 12 hours of observation even exceeded 20 % in all sections.

In total the extra oxygen uptake in the treated sections amounted to 100–150 μl, when I.A.A. was present in the concentration of 1 mg/l.

§ 6. Different concentrations of I.A.A.

No special experiments were carried out to gain information of the I.A.A. concentration which causes the maximal stimulation of the respiration. Usually, it was added in the final concentration of 1mg/l. Only three other concentrations were used, each in one experiment (2 mg/l, 0.5 mg/l and 0.25 mg/l).

The preliminary impression was obtained that the I.A.A. concentration does not affect the duration of the effect, i.e. the stimulation was not prolonged or shortened by increasing or decreasing the I.A.A. concentrations respectively (see Table IX).

From these single experiments at best the preliminary conclusion may be drawn, that the 1 mg/l concentration was not far from optimal.

§ 7. Second addition of I.A.A.

In 6 experiments new I.A.A. was added to the sections at the moment that the effect of the first portion in at least two of three vessels had decreased to zero. So a final concentration of I.A.A. of nearly 2 mg per liter would have been obtained if no I.A.A. had been consumed or inactivated during the previous hours. Subsequent measurements

of the oxygen uptake during at least 6 hours showed that the effect of the new I.A.A. on this process was negligible. Immediately after the addition, in some cases fluctuations of the O_2-uptake were observed; within one or two hours, however, the course of the respiration continued in the original direction as if nothing had happened in between. The average effect of the second addition of I.A.A. did not surpass 1 %, which is insignificant.

§ 8. The influence of ethanol on the oxygen uptake

Up to a few years ago in the Utrecht Botanical Laboratory the I.A.A.-solutions were generally prepared by means of ethanol [1] which was evaporated afterwards. Complete removal of the ethanol, however, cannot be obtained by this method. The remaining negligible small amounts were thought to have no influence on the results when the growth of coleoptiles and coleoptile sections was being measured.

At the present time, the use of alcohol is avoided in our laboratory since the method of solving the crystals in hot water proved not to affect the activity of the growth substance molecules.

It is not known which method is used in other laboratories. In those papers on the influence of growth promoting substances on the respiration, cited in the present publication, no information is given about the procedure.

Apart from a probable influence of small amounts of ethanol on the permeability of the protoplasmic membrane, many cells — among which those of the *Avena* coleoptile (Bonner (1948)) — are able to use this substance as a substrate for the aerobic respiration. Hence, in studies on metabolic changes produced by growth substances, only the second method of preparing the solutions may be applied.

Kelly and Avery (1949) studied the influence of small amounts of ethanol on the respiration of *Avena* coleoptile sections in the presence of abundant sucrose (1 %). Even in this medium an additional increase in the oxygen uptake of 5–15 % was observed. Their experiments do not prove, however, that the increase caused by ethanol was due to an aerobic consumption of this substance since a stimulative effect on sucrose metabolism is not excluded.

A similar restriction holds true for the experiments presented in this section, in which the effect of ethanol on the endogenous respiration and on the I.A.A.-increased endogenous respiration was studied.

In these experiments with starved coleoptiles, which lasted 7 hours, after addition of 40 mg ethanol per liter, an increase in the oxygen uptake over the control of 8–23 % was obtained. In the experiment in which the highest increase was found (23 %), the extra oxygen uptake amounted to 26 μl, which is less than one fifth of the oxygen needed for the complete oxydation of the alcohol present in the vessels. So, for the case that the alcohol is consumed by the cells, the relative small increases point to a small capacity of the alcohol dehydrogenating system, since, under the same conditions, a 100 % increase in

[1] 0.5 ml per 10 ml distilled water for 10 mg I.A.A.

the oxygen consumption was found after addition of glucose (see Chapter II, p. 30).

The following observations were made (Fig. 6) on the influence of ethanol on the increase in the endogenous respiration by I.A.A. At the start of the experiment, ethanol reduced the positive effect of I.A.A. on the endogenous oxygen uptake to about zero. In the next one or two hours, the inhibiting effect of ethanol gradually disappeared so that in the following period no much difference was observable between the quantities of stimulation in the presence or absence of alcohol. In another experiment, however, the final increase in the oxygen uptake caused by I.A.A. in the presence of alcohol was higher than that of I.A.A. alone.

For this reason the question whether the actions of I.A.A. and ethanol on the respiration are independent, additive or even synergistic is hard to answer as the interaction of these substances — if present at all — seems to change in the course of an experiment.

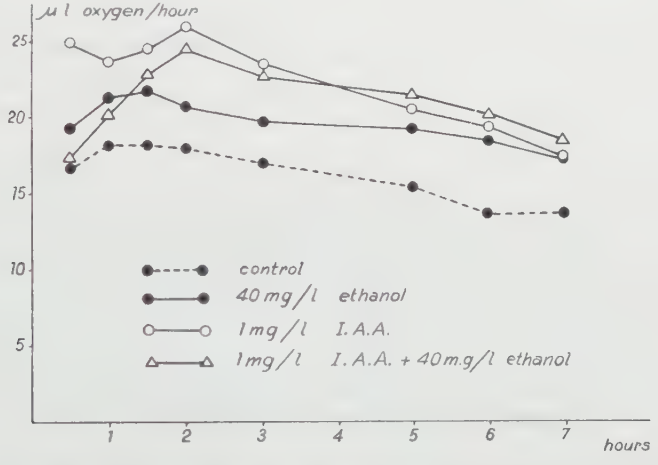

Fig. 6. The effect of ethanol on the respiration and on the I.A.A.-induced increase in the respiration, studied with sections suspended in a substrate-free medium.

It may be remarked, finally, that fig. 6 shows how, in experiments of short duration, the stimulative influence of I.A.A. on the respiration may be masked by the presence of ethanol in the medium. This might provide another explanation why other authors did not find any influence of I.A.A. on the endogenous respiration.

§ 9. Discussion

The experiments of this Chapter leave no doubt about a promoting effect of I.A.A. on the endogenous respiration of *Avena* coleoptile sections. The much smaller effects described in the preceding Chapter, when I.A.A. was added not until 24 hours of starvation had passed, are probably due to long-term unphysiological conditions which presumably reduce the reactivity and increase the variability.

To the same cause it is ascribed that the stimulative effect of I.A.A. is of a limited duration. Higher concentrations do not prolong the effect, more dilute solutions do not shorten it. Nor did addition of new I.A.A. cause a second stimulation of the respiration. These facts strongly indicate that consumption or inactivation of the growth substance do not determine the duration of the effect.

Another potential cause of the ending of the effect is the depletion of the reserve carbohydrates. In both, treated and untreated sections, however, only a small decrease in the starch content could be observed under the microscope when compared with freshly cut sections. In addition, the rate of the endogenous respiration was not much reduced at the close of these long experiments, so that exhaustion of substrate may be excluded too. Consequently, a limited reactivity of the cells is considered the factor determining the duration of the effect.

The stimulation was generally preceded by a varying period of inhibition. The same sequence of effects was observed by Stenlid (1949) when studying the influence of the 2,4-dichlorophenoxy-acetic acid methyl ester on the respiration of wheat roots at pH 7.0, the period of inhibition lasting about 4 hours. This author observed with I.A.A. an inhibition of the respiration at pH 4.6 whereas at pH 7.0 a small stimulation was found after 30–90 minutes, which increased during the subsequent hours.

Significant differences in the reactions of sections of different levels on the coleoptile were not found, except for the observation that the promoting influence of I,A.A. started sooner in the middle sections. It would be of interest to investigate whether the growth response of sections of this level also starts earlier. This suggestion, however, is not based on the belief that the increased oxygen uptake is the result of increased growth, since simultaneous measurements of the growth and the respiration, to be reported in Chapter VI, p. 51 showed that stimulation of the respiration occurs independently of growth reactions.

CHAPTER V

THE EFFECT OF OTHER GROWTH PROMOTING SUBTANCES ON THE ENDOGENOUS RESPIRATION

§ 1. Introduction

In the experiments reported here, the actions of other auxin-like substances were tested in comparison with I.A.A. The effect on the endogenous respiration was followed during 5 to 8 hours with freshly cut sections, representing the 5–7 mm of the coleoptiles, the growth substances being added 3–4 hours after sectioning. As usual, the first hours after sectioning were used to compare the respiration in experimental and control vessels.

The substances tested were: γ-phenyl butyric acid, indole butyric acid, α-naphthyl acetic acid and β-naphthyl acetic acid [1].

The concentrations of the applied growth regulators were equimolar to a 1 mg/l I.A.A. solution.

§ 2. The effects of gamma-phenyl butyric acid, indole butyric acid, alpha-naphthyl acetic acid and beta-naphthyl acetic acid

The results of the experiments carried out with γ-phenyl butyric acid and with indole butyric acid are given in Table X.

TABLE X

Comparison of the percent stimulation of the endogenous respiration caused by indole acetic acid, γ-phenyl butyric acid and indole butyric acid.

Exp. no.	I.A.A.	γ-phen. but. acid	ind. but. acid	duration in hours
1	19	12	7	5
2	20	14	8	5
3	24	11	— [1]	8

[1] = not measured

In Fig. 7 the first experiment is presented.

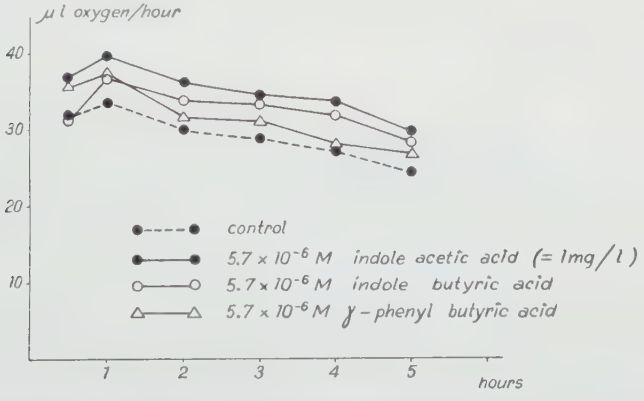

Fig. 7. The effects of indole acetic acid, gamma phenyl butyric acid, and indole butyric acid on the endogenous respiration of sections suspended in a buffer solution.

The results of the experiments with α- and β-naphthyl acetic acid are given in Table XI and in Fig. 8.

[1] The testing of the last substance was suggested by Dr H. Veldstra. For this, and for providing a sample of this substance the author is indebted to him.

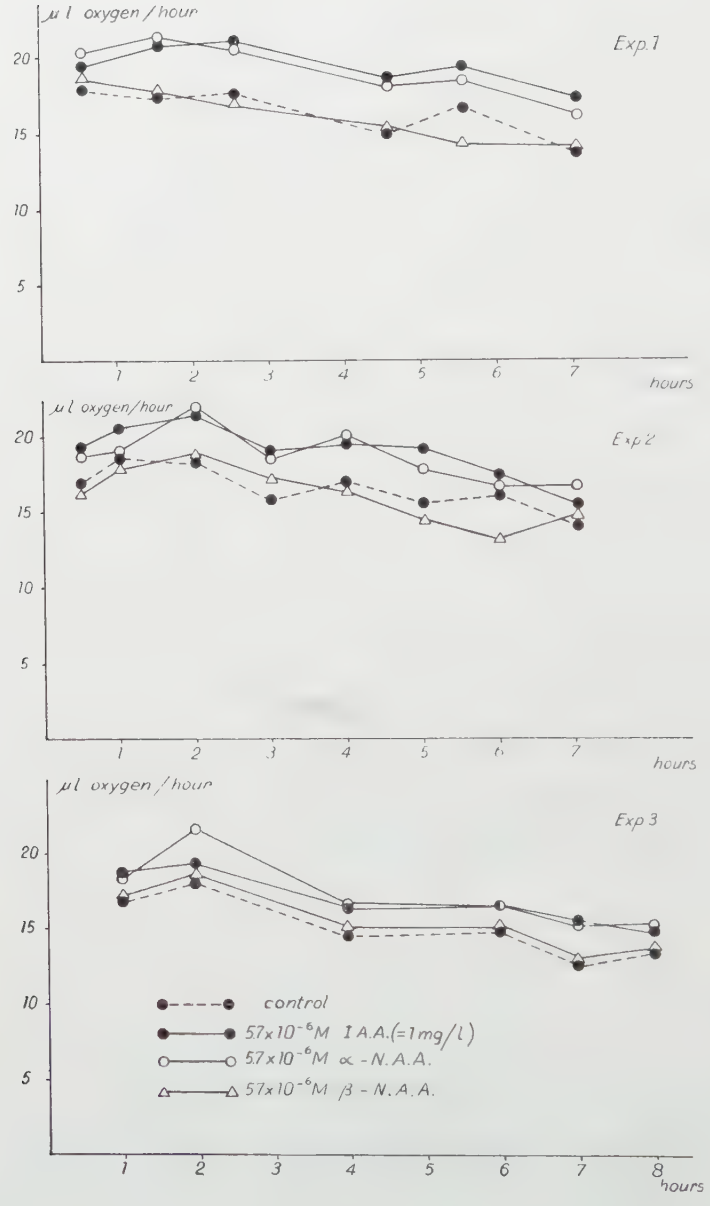

Fig. 8. The effects of indole acetic acid, alpha-naphthyl acetic acid, and beta-naphthyl acetic acid on the endogenous respiration of sections suspended in a buffer solution.

TABLE XI

Comparison of the percent stimulation of the endogenous respiration caused by indole acetic acid, α-naphthyl acetic acid and β-naphthyl acetic acid.

Exp. no.	I.A.A.	α-naphth. ac. acid	β-naphth. ac. acid	duration in hours
1	18	17	— 2	7
2	16	15	— 2	5
3	12	15	+ 4	7

§ 3. Discussion

γ-phenyl butyric acid

According to Went (1939), γ-phenyl butyric acid belongs to a group of substances, which may induce the "preparatory" reaction only and not the growth reaction proper. It is supposed to prepare a physiological condition within the tissue, which causes the effect of certain amounts of I.A.A. on the growth to be larger than on unprepared tissues. γ-phenyl butyric acid, therefore, is one of the so-called hemi-auxins which change the sensitivity of growing cells to auxin. Since the sensitivity of the cells is ascribed by Went to the food factor content, hemi-auxins are thought to be active on the food factor complex. One component of this complex was identified as sugar (Schneider (1938)).

Though it is obvious from Went's experiments that the effect of γ-phenyl butyric acid is not restricted to an action on the sugar supply, it is held that in the present stimulation of the endogenous respiration, this peculiar effect is probably the cause of the phenomenon observed.

The molar activity appears to be about half that of I.A.A.

Indole butyric acid

Contrary to γ-phenyl butyric acid, this substance is active in the growth reaction proper. Except for the standard *Avena* test, the molar activity is practically equal to that of I.A.A. (see Went, 1939-a). The relatively low activity in the standard *Avena* test shows that it lacks one or more secondary properties, since these are supposed to influence the quantity of the curvature.

In the present experiments it appears that the property of activating the endogenous respiration was present to a lesser degree than in I.A.A. and even in the "hemi-auxin" γ-phenyl butyric acid.

α-naphthyl acetic acid

Unlike the former, this substance was equally active in the respiration as I.A.A. Still, its activity in the standard *Avena* test does not differ very much from that of indole butyric acid. This means that, in the case of α-naphthyl acetic acid, inferiority in the standard *Avena* test is caused by another secondary property than that manifesting itself in the present experiments.

Further research along this line with a number of other growth promoting substances will perhaps help in future to clear up the mechanism of action of a given substance on a given process.

β-naphthyl acetic acid

This substance did not stimulate the endogenous respiration. Veldstra (1944) found that the effect on the root formation was about one third of that of the alpha compound and half that of I.A.A.

Zimmermann and Wilcoxon (1935) observed a small activity in causing sweet Pea stems to bend. The alpha compound appeared to be approximately 100 times more active than the beta. They did not exclude the possibility that this small activity was even due to slight contamination with the alpha compound. The present results add to the probability of this supposition.

The importance of testing growth substances on the respiration as a selective test for their activity is shown by the absence of such an effect of beta-naphthyl-acetic acid.

CHAPTER VI

RESPIRATION AND GROWTH

§ 1. Introduction and methods

Earlier in this paper (p. 33) the attention has been drawn to the variability in the response of the endogenous respiration to the addition of I.A.A. Similar quantitative variations were observed by other authors when they measured the growth response of *Avena* coleoptile sections.

The experiments reported here, in which the growth and the respiratory response were determined simultaneously, were designed to detect whether the magnitudes of stimulation of these processes are quantitatively related.

For this purpose plants were selected, of which the primary leaves were about piercing the coleoptiles. This was implied by the fact that in the greater part of the plants the primary leaves had already done so (such individuals were not used). The sections of these coleoptiles were expected to show only a small response to I.A.A.

A second group of experiments was carried out with sections taken from short coleoptiles, the growth response of which is generally larger than that of sections from long coleoptiles.

The growth measurements (determination of the final length of the sections) were carried out with the same sections of which the reaction of the respiration had been determined before. For reasons of comparison it was deemed more exact not to use sections staying under more normal conditions for the growth measurements. As abnormal conditions within the respiratory vessels are to be mentioned the absence of carbon dioxide in the atmosphere and the uninterrupted shaking. Bünning, Haag and Timmermann (1948) showed that

mechanical stimulations caused inhibition of the cell elongation with etiolated seedlings of *Sinapis alba* and of *Vicia faba.*

The sections, being freshly cut, were suspended in a 1 % KH_2PO_4 solution without addition of substrate.

The I.A.A. was added in the final concentration of 1 mg/l. The concentrations of the other growth substances were equimolar to that of I.A.A.

The respiration was measured over a period of 5 to 8 hours, at the end of which the final length of the sections was determined.

§ 2. Simultaneous measurements of growth and respiration

The results of these experiments are summarized in the Tables XII and XIII. The main conclusions are:

1. In experiments with sections taken from *long coleoptiles* the growth of the I.A.A.-treated ones is equal to, or slightly above that of the controls.

2. In all cases a stimulation of the oxygen uptake is found, the percentage of which was independent of the final length of the sections.

TABLE XII

The influence of growth substances on the endogenous respiration and on the growth of sections taken from long coleoptiles

Exp. no.	growth subst.	final length of the controls		final length of treated sections		% stimulation of the respiration
		M	E_M	M	E_M	
1	I.A.A.	56.9	0.6	56.9	0.5	24
2	I.A.A.	61.1	0.3	62.3	0.4	13
3	I.A.A.	58.6	0.3	60.5	0.5	35
4	I.A.A.	59.5	0.3	62.4	0.8	13
5	I.A.A.	59.0	0.5	60.3	0.4	20
	P.B.A.	59.0	0.5	59.1	0.3	14
	I.B.A.	59.0	0.5	59.8	0.7	8
6	I.A.A.	57.6	0.4	58.2	0.7	24
	P.B.A.	57.6	0.4	57.2	0.5	11
7	I.A.A.	60.3	0.5	60.3	0.5	12
	a-N.A.	60.3	0.5	60.2	0.3	15
	b-N.A.	60.3	0.5	59.5	0.5	4

The final length of the sections is expressed in scale units.
The original length amounted to 53 being 3 mm.
I.A.A. was present in the concentration of 1 mg/l, the concentration of the other growth substances being equimolar to this.
P.B.A. = γ-phenyl butyric acid
I.B.A. = indole butyric acid
a-N.A. = alpha naphthyl acetic acid
b-N.A. = beta naphthyl acetic acid
M = mean of 10 sections measured
E_M = standard error

From (1) and (2) it follows that the stimulation of the respiration occurs in the absence of increased growth.

3. In experiments with sections taken from *short coleoptiles* the

growth is increased in the presence of I.A.A., alpha-naphthyl acetic acid and indole butyric acid.

4. The percent stimulations of the respiration are not larger than those observed in non-growing sections of long coleoptiles.

TABLE XIII

The influence of growth substances on the endogenous respiration and on the growth of sections taken from short coleoptiles.

Exp. no.	growth subst.	final length of the controls		final length of treated sections		% stimulation of the respiration
		M	E_M	M	E_M	
1	I.A.A.	58.3	0.7	63.9	0.5	31
2	I.A.A.	56.6	0.2	64.5	0.5	11
3	I.A.A.	60.3	0.3	61.9	0.6	19
4	I.A.A.	57.9	0.5	61.0	0.8	18
	a-N.A.	57.9	0.5	60.2	0.7	17
	a-N.A.	57.9	0.5	56.1	0.4	— 2
5	I.A.A.	60.3	0.5	67.3	0.8	19
	P.B.A.	60.3	0.5	61.6	0.7	12
	I.B.A.	60.3	0.5	66.9	1.0	7

For explanation see under Table XII.

From (1), (2), (3) and (4) it follows that actual growth does not correlate with, in any case does not increase, the stimulation of the respiration.

The percent elongation of the sections from short coleoptiles after addition of I.A.A. varied from 15–25 percent in 5–8 hours.

§ 3. Discussion

The increase in length of 15–25 percent indicates that the conditions for section growth were not extremely bad within the respiratory vessels, since Rietsema (1950), who used the normal cylinder test method with sections suspended in a 0.01 m KH_2PO_4 solution in the presence of 0.1 mg/l I.A.A. observed an elongation of 25–30 percent during the same period of observation. As, however, the concentrations both of the buffer- and of the auxin solutions differed from those used in the present investigation, the results are not quite comparable.

On an average, the stimulations of the oxygen uptake of the growing and non-growing sections were equal. This excludes that the effect of growth substances on the respiration is only the result of the elongation of the sections.

Blank and Frey Wyssling (1941) discovered that an impressive synthesis of protoplasm takes place in Mais coleoptiles during cell elongation. It is an open question whether this part of the growth process can be influenced independently. In the present experiments it is, theoretically, possible that in both growing and non-growing cells the same amounts of new protoplasm (including phosphorylative (?) enzymes) are formed after I.A.A. addition, such at the cost of the reserve nitrogen in the vacuole (see Blank and Frey Wyssling

(1940)). This could account for the fact that the magnitude of stimulation of the endogenous respiration does not depend on the occurrence of growth.

The results, described earlier, however, present evidence that the observed increase in the oxygen uptake is not due to the formation of new enzymes. In several experiments it was found that after the period of stimulation the rates of oxygen uptake in the treated and the untreated sections showed only minute differences or were equal again. This seems to point to a temporary — direct or indirect — stimulation by I.A.A. of the activity of enzymes already present, rather than to a promotion of the formation of new enzymes.

CHAPTER VII

THE INFLUENCE OF INDOLE ACETIC ACID ON THE AMYLASE ACTIVITY IN VITRO

§ 1. Introduction

The experiments reported in this section are a repetition of part of the work of Eyster (1946), Smith, Langeland and Stotz (1947) and Brakke and Nickell (1952). These authors obtained contradictory results when studying the influence of I.A.A. and of other growth substances on the activity of isolated diastases.

In measuring the diastatic activity two procedures can be followed. First, the amount of unchanged starch can be measured at regular intervals by using the iodine staining technique. Secondly, the amount of reducing sugars, formed in this reaction can be determined by titrating the reaction products.

The first method was used by Eyster who measured the time required for the digestion of soluble starch past the last iodine staining stage. All growth regulators appeared to inhibit the diastatic activity, the effect being correlated with the pH of the medium that was not buffered.

Smith, Langeland and Stotz determined the effect of I.A.A. on the diastatic activity by measuring the saccharification rates using the alcaline ferricyanide technique, described by Redfern and Johnston (1938). No stimulation or inhibition was observed.

After the experiments reported in this chapter had been completed, a paper was published by Brakke and Nickell (1952), who studied the influence of growth regulators of different structure on the activity of an alpha amylase secreted by virus tumor tissue from roots of *Rumex acetosa* grown in vitro. The enzyme activity was determined spectrophotometrically, by measuring the decrease in optical density of the starch iodine complex. The experiments were made with different growth substance concentrations but in no case a significant effect was obtained.

In the present investigation both the iodine staining technique and the sugar titration method were tested. It appeared that the first

method was less suitable in this special case, since in the presence of I.A.A. complications occur which may easily lead to incorrect interpretations.

§ 2. The iodine staining technique

To one of two series of Pyrex test tubes containing buffered solutions of 1 % amylum solubile (2½ % KH_2PO_4, pH = 4.5) plus pure alpha and beta amylases (5 mg/l), I.A.A. was added to the final concentration of 10 mg/l. To the other series distilled water was added instead. The progress of the reaction was studied at 22° C. through adding every half hour 0.1 ml of a fivefold diluted Will's solution (6 g KJ + + 2 g J_2 in 120 ml aq. dest.), which caused a blue colour of the test fluid. The rate of starch hydrolysis was measured by determining the time required to get "no blue colour" after addition of iodine, since "no blue colour" means, that all starch has been broken down to final or intermediate products. At the eighth observation (after 4 hours) this occurred in experimental as well as in control tubes, a fact indicating that the reaction rate was not much affected by I.A.A., though — it is admitted — the intervals between measurements were rather long.

The fact that the "no colour" point was reached by experimental and control tubes simultaneously was surprising, since observations during the previous hours seemed to point to an acceleration of the reaction by I.A.A. From the beginning the colour in the experimental tubes was of a less intensive blue than in the controls. As soon as it was noticed, however, that the colour of the experimental solution was not constant and even disappeared after about 8 hours, it became obvious that complications were going on. After adding fresh iodine the colour reappeared.

It is common knowledge that the blue colour of a iodine-stained starch solution can be made to disappear by heating, and that after cooling the colour will return. With I.A.A. present in the solution in ample concentration, the blue colour did not return, the solution remained uncoloured. If, however, iodine was added in excess, the presence of I.A.A. did not make any difference.

This problem was solved by the following experiment. When a solution of I.A.A. + J_2KJ was heated to about boiling point the reaction mixture became turbid. This turbidity appeared at room temperature as well, but at a much slower rate. The precipitation was not analyzed. Addition of starch showed that iodine had disappeared from the medium. From this the conclusion was drawn that iodine had combined with I.A.A.

After these observations the explanation of the colour differences in experimental and control tubes is obvious. The extinction of the colour is due to the disappearence of free iodine from the reaction medium.

These experiments were not continued since, in the meantime, well reproducible results were obtained with the following method.

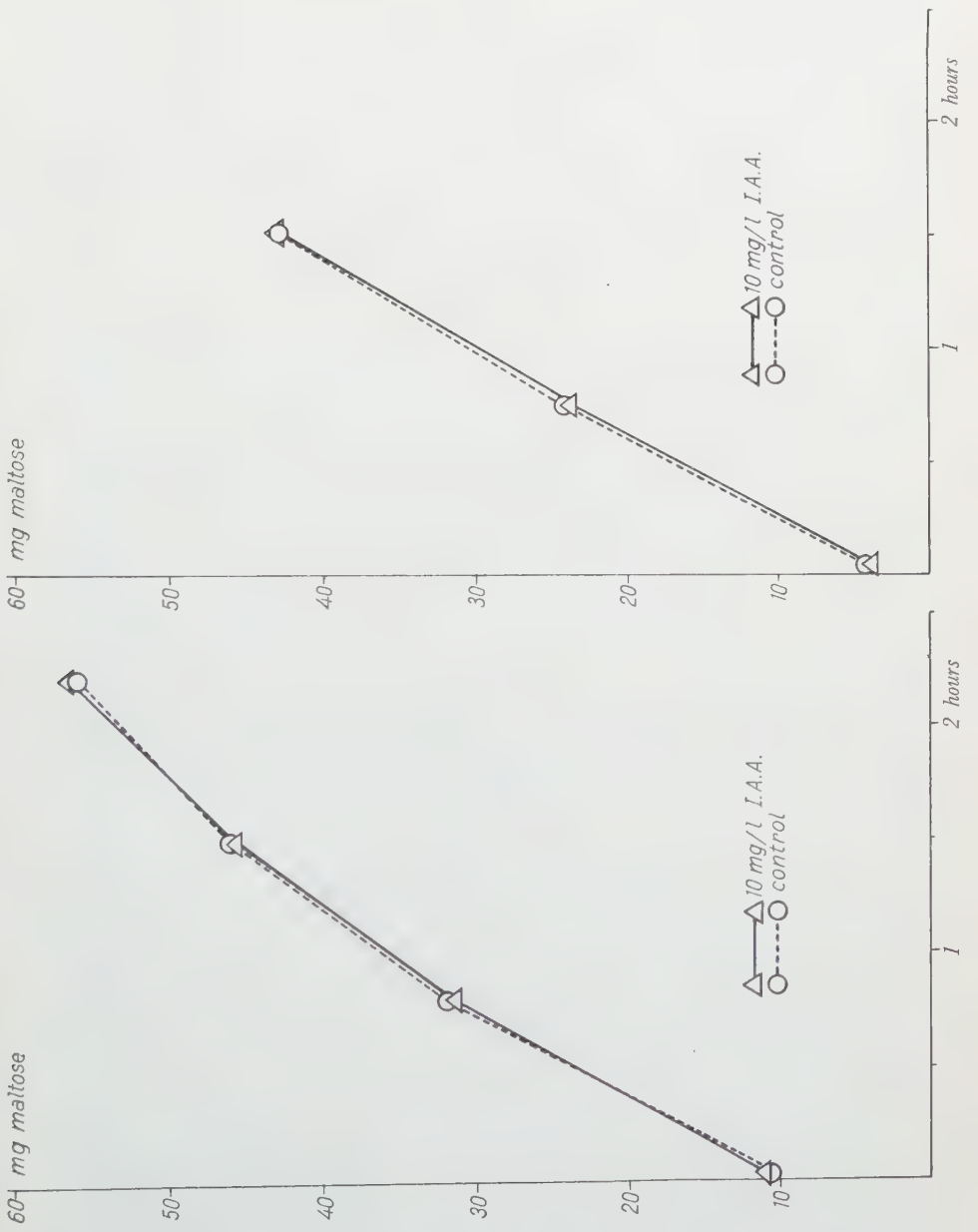

Fig. 9. Absence of effect of indole acetic acid on the amylase activity in vitro.

§ 3. Determination of sugar production

The titration was carried out after the method of Schoorl (1929). It is based on the reduction of cupri- to cupro ions by reducing sugars, the remaining cupri ions being determined iodometrically.

The diastatic activity was studied in a medium containing 0.75 % amylum solubile, 100 p.p.m. I.A.A. and 1–1.5 p.p.m. alpha and beta amylases. The reducing power of the reaction mixture is expressed in mg maltose.

The results show that I.A.A. does not influence the rate of starch hydrolysis by amylase in vitro (fig. 9).

§ 4. Discussion

It seems justified to conclude from the data presented in the literature, which are confirmed by the present experiments, that direct stimulation of the amylases is not the mechanism by which I.A.A. stimulates the starch hydrolysis. There is no special reason to assume that the results of these *in vitro* studies are of less importance for conclusions on the situation in the living cell than similar *in vitro* studies were with other enzymes, such as dehydrogenases etc.

The absence of a direct influence of I.A.A. on the amylases does not exclude a stimulation of amylolytic activity *in vivo* since it may be caused by other mechanisms, for instance, a release of an enzyme from a bound state to an active one. This point will be discussed in the next chapter.

That Eyster nor Brakke and Nickell did notice extinction of the blue colour in the presence of I.A.A. must probably be ascribed to the fact that more concentrated iodine solutions were used, or, that the solutions, after having been observed, were immediately rejected.

GENERAL DISCUSSION

§ 1. Did I.A.A. function as a substrate for the respiration?

Before dealing with the possible mechanism of the I.A.A.-induced increase in the oxygen uptake, the question must be discussed whether this effect might be explained by oxidation of the growth substance itself. Was the extra oxygen, taken up after addition of I.A.A. perhaps used - wholly or partly — in a "respiratory process" in which I.A.A. functioned as a substrate?

This is no purely theoretical possibility, since enzyme systems, which inactivate I.A.A. under oxygen consumption do exist (Tang and Bonner (1947), with etiolated pea epicotyls; Wagenknecht and Burris (1950), with bean roots). In this reaction one molecule of oxygen was taken up and one molecule of carbondioxyde was released per one molecule of transformed I.A.A. Since the end product of this breakdown could be solved in ether and since it had an intact indole nucleus, the latter investigators concluded that indole aldehyde might be the end product.

If the possibility is taken into account that such an enzyme system

also occurs in *Avena* coleoptiles, the question becomes important whether this oxydation might serve as an explanation for the extra oxygen consumption observed after addition of I.A.A. to the sections.

In the experiments described above the effect was caused by an I.A.A. concentration of 1 mg per liter. For oxidation of all molecules, 0.32 μl of oxygen would be needed per vessel. Since, on an average, in the vessels with I.A.A. 5 μl per hour more oxygen was taken up than in the control vessels, and since the stimulation could last more than 20 hours, it is obvious that oxidation of the I.A.A. — if occurring at all — could only account for a negligible part of the effect.

§ 2. Stimulation of the reserve food mobilization by I.A.A.

It has been shown in the second Chapter (p. 30) that (1) the rate of the endogenous respiration of *Avena* coleoptiles is about half that of the exogenous respiration and that (2) this relatively low oxygen uptake is not caused by a reduction in the capacity of the respiratory enzymes under the prevailing conditions of starvation. Since, even after long experiments, no exhaustion of the starch content was observed under the microscope, the conclusion has been drawn that the rate of the endogenous respiration is determined by the rate of reserve food mobilization.

A considerable number of investigations has shown that stimulation of this mobilization must be considered a normal aspect of growth substance action. (Borthwick, Hamner and Parker (1936), Mitchell and co-workers (1937, 1938, 1940, 1945) Reinders (1938, 1942) and many, more recent publications). The products originating from this process were thought to provide material for cell wall formation and for other synthetic growth processes, or to cause water uptake by enlarging the amount of osmotically active material.

Still another possibility was suggested by Reinders (1938, 1942). She observed an increased loss of dry weight when adding I.A.A. to discs of potato tubers. This effect was ascribed to an oxydative breakdown of the starch. Also Rasmussen (1947) suggested an increased utilisation of reserve carbohydrates in the respiration after addition of 2,4-D to roots of *Taraxacum officinale*.

In the author's opinion, the present results have to be explained by the same phenomenon, since the respiration was limited by the substrate concentration. When the latter is the case, no increase in the oxygen uptake may be expected by stimulation of the substrate oxydizing enzymes. The observed increase in the endogenous respiration cannot be explained, therefore, by the theory of Commoner and Thimann (1941, see p. 24), which was based on experiments with ample supply of substrate. It must be emphasized, however, that this inapplicability only concerns the bruto oxygen uptake in the present experiments and not possible qualitative changes in the endogenous respiration. These changes might occur in the *way* of the *consumption* of the mobilized reserve food (for instance a stimulation of the C_4-dicarboxylic cycle).

When the stimulative action of I.A.A. is interpreted as providing

substrates to the respiratory enzymes, the question arises by what mechanism the reserve carbohydrates are made more readily available to the respiration.

§ 3. The mechanism of the effect

If I.A.A. and other growth regulators really affect the starch consumption inside the cells, there are, apart from an improbable direct stimulation of the hydrolyzing enzymes, other possible mechanisms of action. One of these, proposed by Eyster, is a release of the enzymes from a bound state to an active one. This was suggested by results obtained with model experiments, in which growth regulators were found to release diastase from activated charcoal. Smith, Langeland and Stotz (1947), however, repeating this investigation under more rigorous experimental conditions were unable to confirm this result.

Another possibility is that starch inside the cell is surrounded by proteins, as by some investigators glycogen is supposed to be (Willstätter & Rhodewald (1934) and Przylecki & Majmin (1934)). In that case the increased consumption of starch by addition of I.A.A. might be explained by removing the hypothetical protective layer and thus making the starch more accessible to the diastases (see below). Such actions on proteins were already claimed by Northern (1942), who suggested as the primary action of auxins a dissociation of associated protein molecules, which would cause stimulation of diverse metabolic processes, including starch hydrolysis.

The above considerations on the stimulation of the starch breakdown by hydrolysis may be extended to the phosphorylative activity of the cells. These enzymes, which catalyze the combination between phosphoric acid and polysaccharides were found in many parts of higher plants (Hanes, 1940). They give rise to the formation of the Cori-ester (glucose-1-phosphate) which, in case of further oxydative breakdown, undergoes transphosphorylation to glucose-6-phosphate. It is held by Meyer (1943) that with glycogen this is the normal way of breakdown in the cell metabolism, such in contrast to what happens in the digestive tract, where it is attacked by amylases. Possibly, the same holds true for starch when it is involved in an oxydative breakdown.

In recent papers much attention has been paid to the possibility that the primary action of auxins is exercised on the phosphate metabolism.

Bonner and Bandurski (1952) are of the opinion that auxin "must in some way affect the phosphorylative process". Their view is partly based on the assumption that the rate of the respiration — which is increased by auxins — is limited by the phosphorylation and by neither the substrate concentration nor the capacity of the respiratory enzyme system.

With Bonner, Van Overbeek (1952) believes that the organic acid metabolism provides a substrate for auxin action, in the way that by this part of the respiration the high energy phosphates are generated "the transfer or utilisation of which is regulated by auxins".

Although phosphorylation of starch cannot occur without phosphates, it is held that the endogenous respiration is not stimulated by facilitating the phosphate supply. For, it cannot be assumed that in the present experiments with starved sections the phosphate concentration was controlling the rate of oxygen uptake at the moment of auxin addition. Addition of sugars being immediately followed by a rapid increase in the respiration, it is obvious that the amount of free sugars was limiting the rate of oxygen uptake before, and not those agents by which the sugars are involved in the oxydative breakdown (phosphates, phosphorylating and oxydizing enzymes).

At this point of the discussion an argument may be moved, derived from observations reported in Chapter IV § 2. (p. 38). It was found that the stimulative action of I.A.A. was preceded by a period of inhibition, which lasted some 1–2 hours. In terms of phosphate metabolism this would mean an I.A.A.-induced withdrawal of phosphates from the phosphorylative enzymes, the original situation being restored only after one or two hours.

It seems more obvious that in this initial inhibition of the respiration another link in the chain of endogenous respiration is affected by I.A.A. In this connection the attention may be drawn to papers of KONINGSBERGER (1942, 1947).

KONINGSBERGER and co-workers, using isolated protoplasts obtained from mesophyllous tissue of bulb scales of *Allium cepa*, studied microcinematographically the effect of I.A.A. on the change in volume when these protoplasts were transferred from a 1.5 M into a 0.75 M glucose solution. The increase in volume (i.e. the water uptake) was not only delayed for 7–9 minutes as compared with the control, but also markedly retarded over a period of at least 1–2 hours (fig. 10).

With the considerations of VELDSTRA (1944) in mind, these results

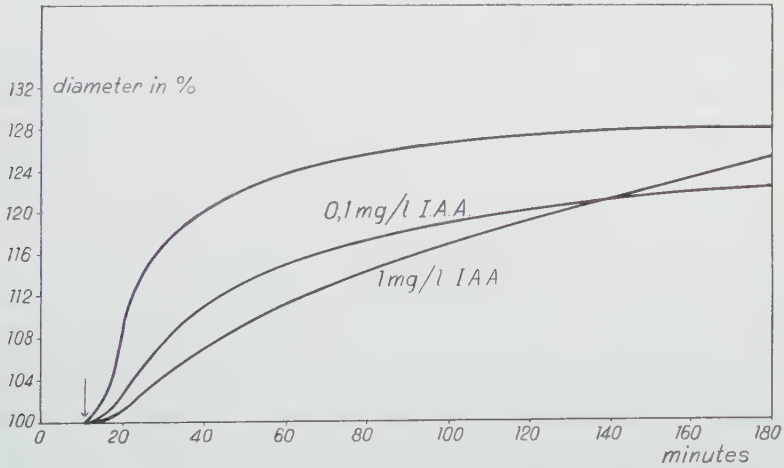

Fig. 10. The effect of indole acetic acid on the increase in volume of isolated protoplasts of *Allium cepa*, when transferred from a 1.5 mol/l to a 0.75 mol/l glucose solution. (after KONINGSBERGER, 1947).

were explained by claiming a condensing effect of I.A.A. on the protoplasmic membrane which caused a decreased water permeability. In some cases the final volume of the I.A.A.-treated protoplasts exceeded that of the controls, which pointed to a decrease in the elastic properties of the protoplasm.

Similar reactions of the protoplasm were described by NORTHEN (1942) as a decrease in the structural viscosity of the protoplasm due to the dissociation of cellular proteins.

The present effect of I.A.A. on the endogenous respiration runs more or less parallel with the results of KONINGSBERGER et al., a positive effect being often preceded by a 1–2 hours period of inhibition. The observed change in metabolic activity, therefore, might be explained in terms of variations in the structure of inner protoplasmic membranes, which increase or decrease the accessibility of enzymes to substrates (in casu starch).

This explanation is completely in agreement with the ideas of BÜNNING (1936) concerning the regulation of the respiratory intensity. BÜNNING, being convinced that the rate of the respiration in plants is, to a high degree, dependent on the substrate concentration, considered the reserve food mobilisation as the rate controlling process of the oxygen uptake. The amylolytic activity, in its turn, would depend on the presence or absence of a spatial separation between the starch and the enzymes. Evidence in favour of the existence of protoplasmic membranes between enzymes and substrates has been reviewed by VON PRZYLECKI (1935) for the case of polysaccharide breakdown.

A similar general influence and not an effect on a special enzyme was implied by STENLID (1949) when he explained the stimulation by 2,4-dinitrophenol on the respiration of wheat roots. As the mechanism of action he suggested a "destruction of some cell structures which leads to increased contact between some respiratory substrate and an enzyme system normally not working with full efficiency. It is known that many organs normally contain both enzymes and the appropriate substrates but that in spite of this no reaction occurs". It has been mentioned earlier that this author too observed a stimulation of the respiration after addition of the methyl ester of 2,4-dichlorophenoxy acetic acid, which was preceded by a period of inhibition.

It will be clear that the present experiments with *starved Avena* coleoptile sections were not intended to check the validity of the theories of THIMANN *et al.*, BONNER and BANDURSKI and of VAN OVERBEEK.

The above theoretical considerations, therefore, only mean to search for an explanation for those cases of stimulation of the respiration, in which the other theories do not seem applicable.

The fact that gamma phenyl butyric acid, a substance with a negligibly small effect on the growth reaction proper, is active on the endogenous respiration, proves that auxin action includes more than what has been studied here, and what might be best considered as an aspect of, what WENT called the preparatory reaction. (Chapter V, § 3, p. 49).

WENT (1939) believes that in the preparatory reaction growth substances have a positive effect on the upward transport of certain substances, needed for cell elongation (food factor).

In isolated sections, suspended in a substrate free medium, however, such factors cannot be supplied by transport. Consequently, their growth, which is considerably increased by I.A.A., is dependent on the reserve substances. From this it follows that in this case at least, part of the food factor complex is mobilized on the spot. Since, even in freshly cut sections the respiration is markedly increased after addition of sugar (which points to substrate shortage), it is not unlikely that the above also holds true for intact coleoptiles. By the presence of large quantities of starch this probability is still increased.

It may, therefore, be tentatively concluded that the preparatory action of growth substances is of a physico-chemical nature, being exercised on protoplasmic interfaces. It is not unlikely that also changes in such membranes may increase the upward transport of substances, needed for the growth of intact coleoptiles.

This is not the place for broad discussion on the probability that the growth reaction proper and other effects (increased cambium activity, root formation, etc.) are caused by the same mechanism. However, a few remarks may be made.

Since the manifold chemical reactions occurring in the hydrophylic protoplasm do not proceed chaotically, it is not improbable that the loci of these reactions are separated and that the reactions are regulated by structures which are of a different nature (lipophilic). As there is no reason for assuming that these lipophilic films are all of exactly the same structure, one may suppose that growth substances regulate the cell activity as a whole, as a consequence of different affinities to these films. According to this idea the effect of different concentrations of growth substances (optimum curve for each type of reaction as well as the manifold final effects, dependent on growth substances concentration) seems to become more easily conceivable.

The weakness of implying such general actions on the protoplasmic organisation is, however, that it is not sufficiently supported by clear-cut experimental evidence.

SUMMARY

1. By means of the Warburg manometric technique some details were studied of the respiration of starved *Avena* coleoptile tissue.

2. For this purpose the oxygen uptake was measured on 3 mm sections cut from different levels on the coleoptile (5–7, 8–10 and 11–13th mm from the tip). Instead of being thoroughly mixed — a procedure followed by other authors — the sections from a given position on the coleoptile were studied separately.

3. The amount of oxygen taken up per section per hour showed a slight increase in the basal direction of the coleoptile.

4. Addition of glucose to starved sections caused an increase in

the oxygen uptake of an average of one hundred percent. This is considered an indication that the respiration of such sections is limited by the substrate concentration.

5. The intensity of the endogenous respiration showed diurnal fluctuations. Arguments were moved to connect this with diurnal fluctuations in the rate of starch mobilization.

6. Addition of indole acetic acid (1 mg/l) to sections, starved over a period of 24 hours, generally caused an increase in the oxygen uptake.

7. The respiration of sections of different positions on the coleoptile showed quantitative differences in the response to indole acetic acid addition. The zone of maximal sensitivity, though generally situated between the 5–10 mm from the tip of the coleoptile, in some cases was found at the level of the lowest sections.

8. In experiments with freshly cut sections, the indole acetic acid being added within three hours after sectioning, without any exception, considerable stimulations of the oxygen uptake were found. The stimulations were preceded by a period of inhibition, the duration of which varied according to the position of the sections in the original coleoptile.

9. The stimulative effect of indole acetic acid on the oxygen uptake of freshly cut sections lasted nearly 20 hours on an average. The total extra oxygen uptake in the treated sections amounted to 100–150 mm^3, when indole acetic acid was present in the concentration of 1 mg per liter.

10. Addition of new indole acetic acid to the sections at the end of the period of stimulation was not followed by another increase in the oxygen uptake.

11. Addition of ethanol in a concentration as usually applied in dissolving growth substances (40 mg/l) increased the oxygen uptake of freshly cut sections by 8 23 percent. The presence of ethanol influenced the stimulation caused by indole acetic acid.

12. The action of other growth promoting substances was compared with that of indole acetic acid. Whereas the response of the endogenous oxygen uptake to alpha naphthyl acetic acid appeared to equal that of indole acetic acid, beta naphthyl acetic acid had no influence at all. The activity of gamma phenyl butyric acid being about half that of indole acetic acid, even exceeded that of indole butyric acid.

13. The degree of the stimulation in non-growing sections (cut from very long coleoptiles) was not less than in growing sections. The increase in oxygen uptake, therefore, was not the result of increased growth of the sections.

14. In accordance with data from the literature no effect of indole acetic acid was observed on the hydrolysis of starch by amylases *in vitro*. The unsuitability of the iodine staining technique for such investigations was demonstrated, as indole acetic acid combines with the iodine.

15. It is suggested that the auxin action, underlying the increased endogenous oxygen uptake, is exercised on inner protoplasmic mem-

branes (lipophilic interfaces) thus making the reserve food more accessible to the metabolic processes.

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THE ARRANGEMENT OF THE VASCULAR BUNDLES IN THE NODES OF THE DIOSCOREACEAE

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With plates I–II

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Introduction

In 1926 Mason (1) drew the attention to the fact that in the nodes of the stem of the *Dioscoreaceae* special xylem and phloem structures are found which join respectively the xylem and phloem elements of the vascular bundles running in the successive internodes. He called these structures wood plexus and phloem plexus. Especially the phloem plexus was further described by him.

Mason's results can be briefly summarized as follows. The sieve tubes of the successive internodes do not join up with each other directly, but via a glomerulus, a great number of oblong thin-walled parenchymatous cells running fairly parallel, with a distinct nucleus and densely plasmatic contents. These glomeruli are connected with the ends of outgoing and incoming sieve tubes by similar cells (bast tubuli) lying less compactly and being according to the distance to be bridged, of different lengths. On the ground of this anatomic structure, Mason concludes to a secretory function of the glomeruli, thinking it at the same time probable that these glomeruli form an obstacle for a rapid moving of carbohydrates along the phloem. Whereas, therefore, a mass transport in the internodes through the sieve tubes is conceivable, the substances transported through the phloem must pass the plasm at the nodes. An analogous case Schumacher (2) sees in the anatomical relations, as they appear in the haustorial connections of the parasite *Cuscuta odorata* on *Pelargonium zonale*.

Roeckl, quoted by Esau (3) also examined the structures in the *Dioscoreaceae* and her conclusion was that their anatomic structure did not differ sufficiently from the normal phloematic tissue, to justify Mason's speaking of non phloematic tissue in this case. Happ, quoted by Huber (4) speaks in this connection of sieve cells, verbatim: "reich getüpfelten, aber keine Siebplatten führenden Parenchymzellen (wohl richtiger Siebzellen)".

In connection with the theoretical importance of this matter, a description of the anatomical structures observed by me, may be useful.

The course of the vascular bundles in a number of *Dioscoreaceae* has been examined at the hand of free-hand and microtome sections. The free-hand sections made of the fresh material were stained in coralline soda, according to STRASZBURGER's (5) directions, whereas the microtome sections, in imitation of MASON were stained in haematoxyline. As evidence all particular structures described were fixed on photographs. The research was performed in the Botanical Laboratory of Groningen, Department Systematic Botany, director Prof. Dr R. v. d. WIJK.

ANATOMICAL INVESTIGATIONS

In the internodes in the outer layers of the central cylinder run a number of vascular bundles, arranged in a circle. We find always one opposite a rib and one or two between two ribs. The number depends on the species examined and in the species on the thickness of the stem. The vascular bundles opposite the ribs pass at the nodes into the petiole, that is three per leaf, the others continue uninterrupted. If more than one leaf is inserted at one node (binary, ternary and sometimes quaternary false whorls may occur side by side on the same stem) all vascular bundles opposite the ribs may leave the stem. The relation between phyllotaxis and course of the vascular bundles was extensively described by QUÉVA (6).

The leaf traces leave the stem at the nodes by curving outwards about rectangularly and disappearing into the petiole in that way. The wood parenchyma, which encloses the xylem vessels, and the sclerenchyma, which encloses the phloem vessels, is developed more strongly locally, so that a picture arises as has been given schematically in Fig. 1.

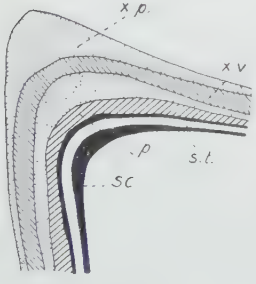


Fig. 1. Schematic drawing of the arrangement of a vascular bundle passing into the petiole. x.p. xylem parenchyma s.t. sieve tubes x.v. xylem vessels p. parenchyma sc. sclerenchyma

At the nodes the number of vascular bundles vanishing into the petiole are supplemented by branching. Besides that supplement this branching also supplies the vascular bundles going into the axillary buds. Per leaf about one third of the total number of vascular bundles found in the stem, participate in the branching, so that of a ternary whorl all vascular bundles are involved in the branching. Now this branching has a special character in the *Dioscoreaceae*. In a longi-

tudinal section of the stem, made in such a way that the petiole is about cut in halves, there is in the stem just below the leaf insertion a suboval complex tissue, macroscopically visible. This complex consists of tracheids, running in all directions, among which especially at the base and at the top in open spaces, conglomerates of oblong parenchymatous cells are conspicuous. At the base the vascular bundles disappear into this complex, at the top they emerge from it, the vascular bundles to the axillary bud arising from it too. The complex itself is formed by repeated splitting up of xylem and phloem elements into elements getting narrower and narrower, next uniting again and so forming the vascular bundles of the next internode. In principle this branching runs parallel for xylem and phloem; this is clearly shown in the schematic figures 2 and 3.

On first considering the xylem (Fig. 2), we note that the course of things is as follows. The vessel running in an internode (v_1) splits up

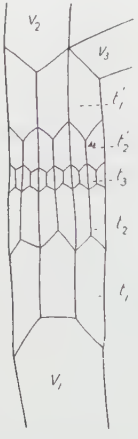


Fig. 2. Schematic drawing of the splitting up of a wood vessel via a complex of tracheids into two vessels, one of which remains in the stem (v_2) and the other going into the axillary bud (v_3). t_1 and t'_1 tracheids of the first order, t_2 and t'_2 tracheids of the second order, t_3 tracheids of the third order.

at the end in three (in reality in a varying number) tracheids of the first order (t_1). These tracheids of the first order pass in a corresponding way into tracheids of the second order (t_2), these again into tracheids of the third order (t_3). It is difficult to trace how often this splitting up is repeated, but finally it takes place in a reverse direction and we get through a confluence of a number of tracheids of a higher order, tracheids of a lower order, so $t_3 \rightarrow t'_2 \rightarrow t'_1$. These last pass into the vessels for the next internode (v_2) and for the axillary bud (v_3).

Of course things are greatly simplified in this scheme. In fact the splitting up at the end of v_1 does not only take place in the longitudinal axis but also laterally. Owing to this the branching of the xylem elements gets a very erratic character and looks like an intricate complex of tracheids, in which there also occur interconnections between tracheids originating from vessels of neighbouring vascular bundles. Where the tracheids are not adjoining, they are surrounded by wood parenchyma (living contents, starch grains).

At the hand of a number of photographs (Plate I–II) we can now imagine the exact arrangement. Photograph A shows a vessel of a vascular bundle of the stem splitting up just below the complex into two tracheids of the first order. These tracheids split up in the same way into tracheids of the second order, which is shown in photograph B. The partition walls between vessels and tracheids and between tracheids among themselves, have besides greatly thickened wall parts also non-thickened ones, which give us a very strong impression of their being perforations. In photograph C we see at the end of a vessel, the partition walls from a number of tracheids. This gives us a clear picture of the structure of the transverse walls. Photograph F (1) gives a survey of a part of a wood complex, from which the erratic arrangement of the tracheids clearly appears. The partition walls between wood parenchyma and tracheids bear bordered pits or have net-like thickenings (photo D).

Mutatis mutandis the branching of the phloem elements has taken place in the same way (Fig. 3). As a rule the splitting up takes place

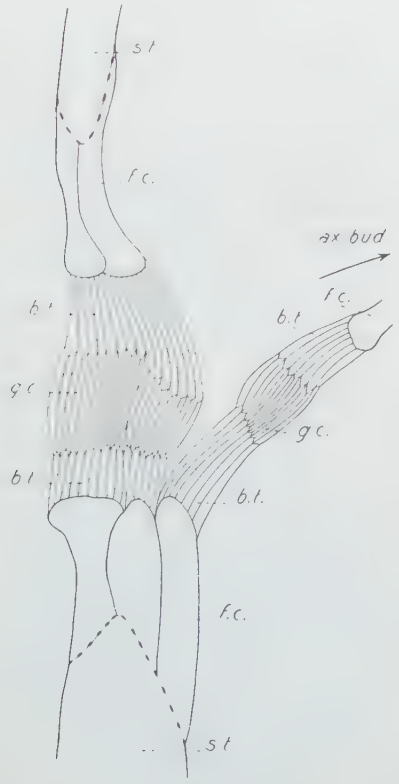


Fig. 3. Schematic drawing of the splitting up of a sieve tube via glomeruli into two sieve tubes, one of which remains in the stem and the other going into the axillary bud.
s.t. sieve tubes, b.t. bast tubulus cells, f.c. funiculus cells, g.c. glomerulus cells.

three times in this case. The elements formed by splitting up and confluencing are called in imitation of MASON: sieve tubes → funiculus cells → bast tubuli → glomerulus cells → bast tubuli → funiculus

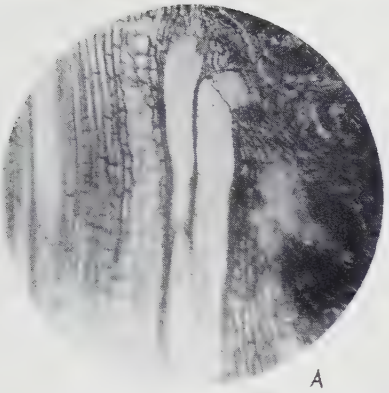
cells → sieve tubes, respectively. As contrasted with the xylem the arrangement is much less erratic. The cells which arise from the end of a certain element, run close together all in the same direction. A sieve tube passes into 2 or 3 funiculus cells, whereas each of these latter pass into a great number of bast tubuli. The length of the bast tubuli varies much and is dependent on the distance to be bridged between two sieve tubes that are being connected with each other. Each bast tubulus is connected with one to three glomerulus cells. While on splitting up each of the successive elements grows narrower and narrower, the total sectional surface becomes much larger owing to the great number. In my estimation the average sectional surface of a glomerulus (i.e. the total of the glomerulus cells, inserted between two sieve tubes merging into each other) is five to ten times larger than that of the sieve tubes joined by this glomerulus.

This arrangement has also been fixed in a number of photographs. Photo E shows, how a number of sieve tubes, running between two xylem parts, pass into glomeruli. In photo F (2) we see, higher magnified, how a sieve tube splits up into three funiculus cells. In the partition wall the sieve plate with thicker and thinner parts is distinguishable. The thicker parts are the sieve fields, the thinner ones the wall parts between them. A view of such a sieve plate from above, as printed on photo G, clearly shows that a single sieve field is also porous; the lighter parts consist of callus (coloured with coralline soda), which has been formed round sieve pores visible among them as dark patches. The funiculus cells originated in this way from a sieve tube end at the other extremity in a club shaped part.

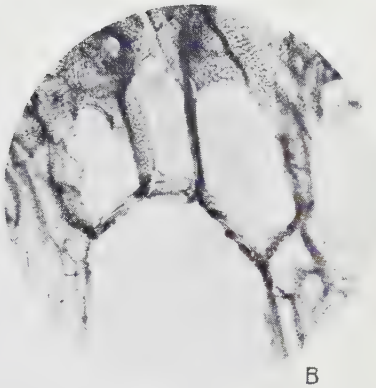
As regards contents, they are not to be distinguished from the sieve tubes. In the wall of the club shaped end more sieve fields occur. To this the bast tubuli are joined, one per sieve field, sometimes one per two sieve fields. Photograph H shows this, while photograph K, more magnified, shows the connection of a bast tubulus with a sieve field. Both the basttubuli and the glomerulus cells have thin walls, being densely filled with protoplasm and possessing a distinct big nucleus. Seeing the fact that the terminal walls are slightly slanting, we had better speak of prosenchyma than of parenchyma (7). The partition walls between bast tubuli and glomerulus cells show besides thin parts also thickened patches provided with callus. The older the

EXPLANATION OF PLATE I

A. Splitting up of a wood vessel into two tracheids of the first order.
B. Splitting up of a tracheid of the first order into three tracheids of the second order.
C. Three partition walls between tracheids seen from above.
D. Partition wall between tracheids among themselves and relief figures on the wall of the wood parenchyma.
E. Two sieve tubes passing into glomeruli.
F. 1. The wood plexus.
   2. A sieve tube passing into three funiculus cells.



A



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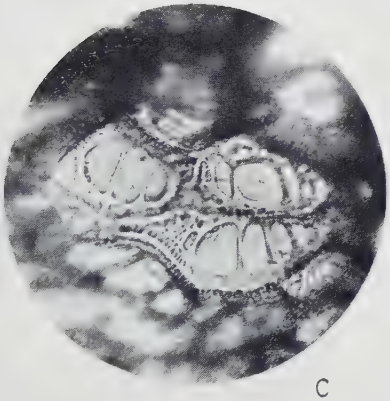

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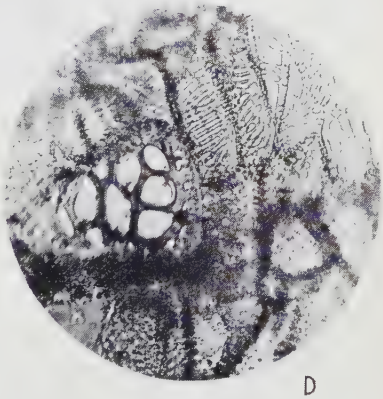

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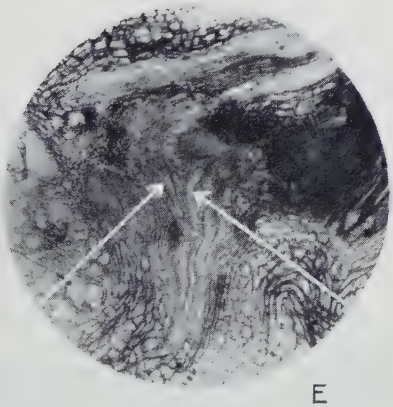

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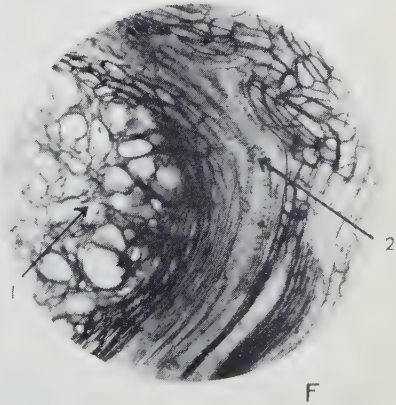

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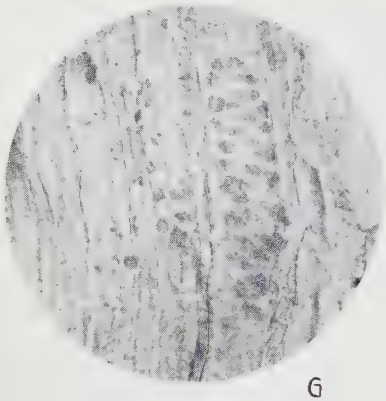

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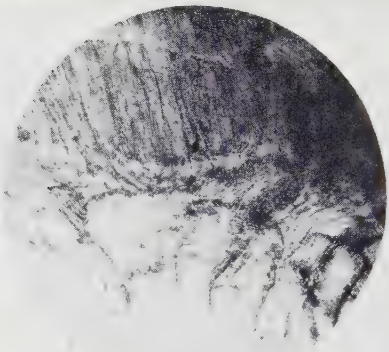

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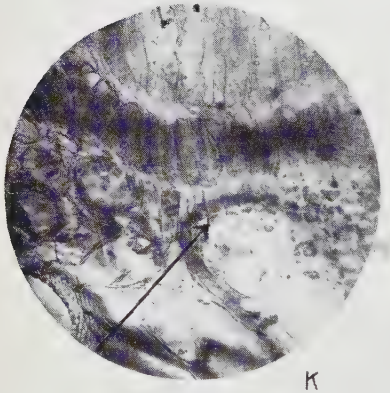

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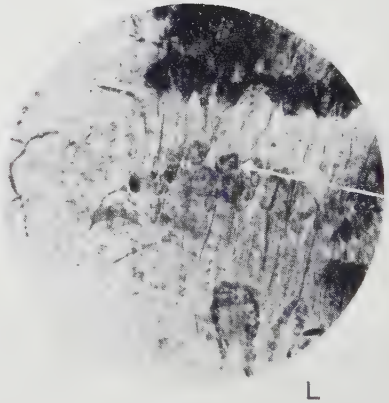

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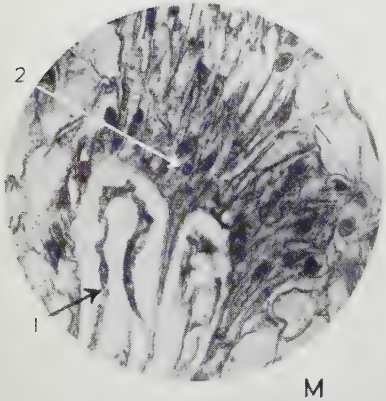
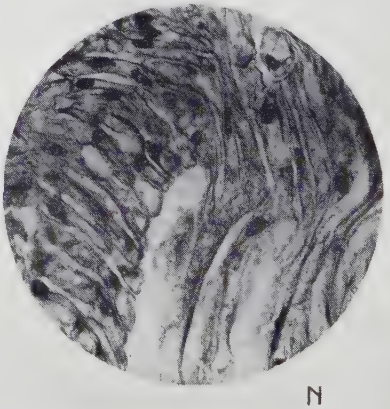


M



N

tissue, the stronger these thickenings come to the fore. This is shown by photograph L. The difference in contents between sieve tubes and funiculus cells on the one hand and bast tubuli and glomerulus cells on the other hand, is clearly shown by the photographs M and N, made of microtome sections after staining in haematoxyline. Whereas in sieve tubes and funiculus cells the thin layer of parietal protoplasm present has coagulated after fixation to an insignificant quantity (M_1), bast tubuli and glomerulus cells are densely filled with protoplasm (M_2). The sieve tubes and funiculus cells lack any trace of a nucleus, whereas in the bast tubuli and the glomerulus cells the nucleus is quite evident (photographs M and N).

Discussion

The above corroborates the facts described by Mason; besides a description of the xylem complex has been added and a further detailed description of the phloem complex. It appears that the occurrence of the complexes described is closely connected with the phyllotaxis.

A wood complex as described above, is not general in the plant kingdom. While in various plants tracheids occur in the xylem by the side of vessels, they usually behave independently of each other in branching. Yet this is not an isolated case. Rouschal (8) describes the same phenomenon for some grasses. There too vessels occur in the internodia, while on the nodes a complex of tracheids joins the vessels of two successive internodes. Meyer (9), who examined the vascular bundles of a great number of plants, states that in various species, the vessels running in an internode, just before or in the nodes pass into tracheids and here too, if not as such a complex tissue, the tracheids form the link between the corresponding vessels in the successive internodes. For the rest it appears from Meyer's publications that the difference between vessels and tracheids is not so great as is frequently assumed. As to the perforations of the walls and the size of the apertures in the transverse walls, every transition occurs between vessels and tracheids. The difference is, therefore, merely quantitative. Eames (10) mentions that sucking Indian ink through the tracheids clearly shows the quite open perforations in the partition walls.

EXPLANATION OF PLATE II

G. A sieve plate consisting of a great many sieve fields.
H. Transition of three funiculus cells into bast tubuli.
K. Connection of the bast tubuli with the sieve fields.
L. Thickened and thin parts in the partition walls between bast tubuli and glomerulus cells.
M. Transition of two funiculus cells into bast tubuli.
   1. Little protoplasm, no nucleus in the funiculus cells.
   2. Much protoplasm, distinct nucleus in the bast tubuli.
N. See M.

A, B, C, D, E, F, G, H, K and L have been made of free-hand sections stained with coralline soda.
M and N have been made of microtome sections stained in haematoxyline.

So it appears that a wood complex as occurs in the *Dioscoreaceae*, is also to be found in other plants and besides that the transition of vessels into tracheids (at the nodes) is no exception.

This does not hold for the phloem complex. As far as is known the occurrence of it is restricted to the *Dioscoreaceae*. It is essential whether there is a qualitative distinction between bast tubuli and glomerulus cells on the one side and sieve tubes and funiculus cells on the other side; a decision, therefore, in the controverse MASON ROECKL. The grounds on which ROECKL and HAPP based their conclusion are unknown to me. MASON did not describe the thickenings in the wall, occurring on the partition walls between bast tubuli and glomerulus cells and consisting of callus (photo L); these indeed suggest phloem tissue. The only important difference between these elements and the sieve tubes which remains, is the contents of the cells. In order to give an opinion on the value to be attributed to this difference, it is necessary to examine the way of differentiation of the sieve tubes. Sieve tubes are differentiated from oblong parenchymatous cells. The initially very abundant plasma contents gradually disappear and a large vacuole is formed. Finally only a thin layer of parietal protoplasm is left and also the nucleus obliterates. Some investigators think that only after this maturing process is completely finished, the sieve tubes can perform their function, transport of assimilates, etc. This may be correct as long as mass streaming is assumed to be the only transport mechanism, but this is by no means probable. ARISZ (11) indicates that before this maturing is completed a specialized parenchyma transport should certainly be considered a possibility. Also the wall of the sieve tubes experiences considerable changes during the maturing process. The sieve plates develop, varying with the species, on the slantwise or oblique end walls and sometimes on the lateral walls of the sieve tubes. The plasm connections through these sieve fields are of the same nature as plasmodesms only much larger. On the thicker wall parts between the plasm connections callus is formed. In course of time this callus formation gets thicker and wider, so that consequently the plasm connection is finally being tied off. After complete closure the sieve tubes stop of course their functioning.

In the growing stem we find from top to base an increasing differentiation. In sections of the youngest nodes, the sieve tubes were already completely differentiated, i.e. the contents had been reduced to a thin layer of parietal protoplasm and the sieve plates were already clearly provided with callus. Of the appertaining bast tubuli and glomerulus cells the partition walls were slightly provided with callus (slightly stained in coralline soda), but marked differences between thickened and non-thickened wall parts were not there.

The contents were densely plasmatic and in each cell there was a clearly visible nucleus. In the older elements the callus deposit is more marked and also the perforation of the transverse walls is clearly visible. The contents, however, remain the same.

Seeing that the substances transported through the sieve tubes along the stem must pass the glomeruli in the nodes and consequently

have to pass through the plasm, a mass transport is surely impossible there. Whether this means a considerable inhibition of the rate of transport is not easily decided. In this connection we do well to consider that simultaneously with introducing "protoplasmic resistance" the track is considerably widened.

So the conclusion is that bast tubuli and glomerulus cells anatomically have a structure strongly departing from fully differentiated sieve tubes. For the transport through this system important consequences are connected with it.

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ABSORPTION AND TRANSPORT BY THE TENTACLES OF DROSERA CAPENSIS

V. INFLUENCE ON THE TRANSPORT OF SUBSTANCES INHIBITING ENZYMATIC PROCESSES

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In the preceding publications absorption and transport by the tentacles of *Drosera capensis* has been analysed. The last two communications having been published in Dutch in 1944, the contents will be given a little more extensively in this paper. The researches have been continued after 1945, but owing to the limited plant material and the fact that the experiments require a great deal of time, the results have not yet been closed. In the first part of this publication, some results of the two preceding communications will be discussed and new data will be added, after which data on the influence of inhibitors on the transport process will be dealt with.

I. Method

Method of cultivation. All experiments have been made with leaves of *Drosera capensis L.* The plants were cultivated in a hot house in earthenware bowls (diameter 20 cms, height 6 cms) divided into 3 parts by two vertically placed glass plates. In the middle space some six Drosera plants grew on ground peat dust. In the side spaces water was poured.

Variability of the experiments. The experiments were made with six series of six leaves taken from six plants of the same age. Each series was composed of six leaves of different ages, one of each plant. In this way the variability of the series was made as slight as possible. Young plants with not too large leaves give the best results. Combining leaves of various ages into one series, however, presents difficulties for metabolic experiments, because age may influence metabolism, as for instance Thimann (1949) showed for the growth of oats coleoptiles. A few weeks after six leaves have been taken from the plants, they are again fit for use for fresh experiments.

Arrangement of the experiments. The experiments were arranged as follows (Arisz and Oudman 1937). From agar layers about 7 mms thick, strips were cut of about 7 mms width and 5 cms length. Two of such

strips were placed on a glass plate in such a way that they were a little further apart than the width of the leaf. A leaf was placed with the marginal tentacles on the two agar strips so that the leaf blade was quite clear of the agar strips and likewise of the glass plate. Next a strip of glass was placed lengthwise across the middle of the blade and firmly pressed into two bits of plasticine lying at the ends of the glass plate (Fig. 1). Finally two strips of agar of the same shape as the first were placed on the marginal tentacles, so that they lay between the strips of agar and were capable of absorbing the substance dis-

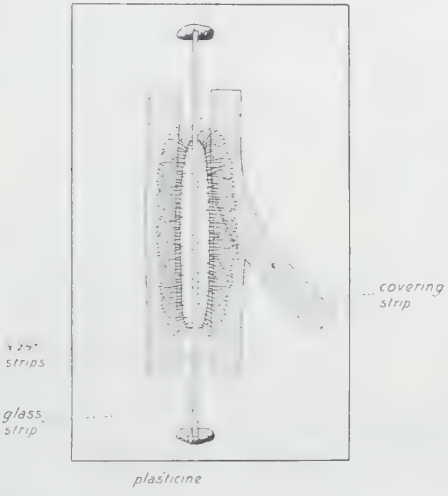

Fig. 1. Leaf of Drosera capensis with the marginal tentacles between strips of agar in which substances have been dissolved.

solved in the agargel. On either side about 80 to 160 tentacles were in touch with the agar. Besides the substance to be transported other substances, such as inhibitors, antibiotica and sugar may be solved in the agar in the concentration required.

Keeping quiet the tentacles. The tentacles, which are stimulated by amino acids, phosphates and similar substances try to curve, in consequence of which they would lose contact with the agar. To prevent them from curving, various methods have been used. Initially caffeine was added, which inhibits curving, when in a sufficiently high concentration. It inhibits, however, absorption as well (Table 1). Keeping the tentacles quiet may also be attained by osmotic dehydration. For this purpose sugar is added to the agar. A 0.30 M sugar concentration had a sufficient osmotic effect to decrease the turgor in the tentacles to such a degree that they remained quietly between the agar strips during the experiment. In later experiments a saltmixture has been occasionally used. We used a mixture of 1.1 gr. KCl + 0.22 gr $CaSO_4$/100 ml. This is isotonic with 0.26 M sucrose. The concentration of the sugar or the saltmixture should be kept as low as possible, because in higher concentrations osmotic actions take place, having an inhibitory effect on the active transport (table 2). To what extent

sugar is taken up by the tentacles was discussed in a previous publication (ARISZ III 1944). The result is uncertain. Reducing sugar increases but at the same time the starch contents of the leaves decrease. It is possible that the influence of the sucrose is indirect and starch is converted into sugar by osmotic influence.

TABLE 1

Simultaneous uptake of phosphate and caffeine. Uptake 48 hours of 1/200 M KH_2PO_4 with addition of sucrose to the agar and of increasing concentrations of caffeine.

	Increase of P_2O_5 per 1000 parts of fresh weight
1/200 M KH_2PO_4 + 0.32 M sucrose	0.51
1/200 M „ + 0.32 M „ + 1/80 M caffeine	0.26
1/200 M „ + 0.32 M „ + 1/40 M „	0.24
1/200 M „ + 0.22 M „ + 1/10 M „	0.08

TABLE 2

Influence of addition of sucrose and of a salt mixture on the uptake of 1/200 M KH_2PO_4 during 48 hours.

	Increase of P_2O_5 per 1000 parts of fresh weight
1/200 M KH_2PO_4 + 0.28 M sucrose	0.83
1/200 M „ + 0.32 M „	0.90
1/200 M „ + 0.36 M „	0.83
1/200 M „ + 0.40 M „	0.53
1/200 M KH_2PO_4 + 0.10 M KCl + $CaSO_4$	0.50
1/200 M „ + 0.12 M „	0.12
1/200 M „ + 0.14 M „	0.02
1/200 M „ + 0.16 M „	—0.11

Sterility. Sucrose moreover has the drawback of promoting the development of bacteria. Though this is not a serious objection in many experiments, it may be important on examining organic substances like amino acids and their amides, or urea, thiourea, phenylurea, etc. It has been investigated whether addition of an antibioticum to the sugar containing agar could prevent the development of bacteria. It appeared that p. amino benzene sulfonamide in a concentration of 0.05 % did not yet delay the uptake and kept the development of microorganisms within reasonable bounds (table 3) Penicillin and streptomycin inhibited the uptake of some transport substances and were therefore unfit for use. To the action of penicillin we shall revert. A disinfectant as germisan was toxic and therefore likewise unfit for use to obtain sterility. Exposure to ultraviolet light did not give a solution either. Therefore the experiments were made with sugar, to which aminobenzene-sulfonamid was added and the

results were compared with those of experiments in which the salt-mixture had been added to the agar.

TABLE 3

a. Influence of addition of sulfonamid on the uptake of phosphate during 48 hours, *b.* uptake of thiourea, urea and phosphate during 24 hours.

a.	Increase of P_2O_5 per 1000 parts of fresh weight
1/200 M KH_2PO_4 + 0.32 M sucrose	0.88
1/200 M „ + 0.32 M „ + 0.02 % Sulfonamid	0.82
1/200 M „ + 0.32 M „ + 0.03 % „	0.88
1/200 M „ + 0.32 M „ + 0.05 % „	0.83

b. Addition of	Uptake of 1/20 M thiourea Increase of N	1/80 M urea Increase of N	1/100 M KH_2PO_4 increase of P_2O_5
0.35 M sucrose	0.62	0.37	
0.15 M KCl + $CaSO_4$	0.60	0.35	0.48
0.35 M sucrose + sulfonamid . . .	0.62	0.34	0.52
0.15 M KCl + $CaSO_4$ + sulfonamid	0.60	0.34	

Mucilage secretion. The glands of the tentacles secret mucilage and this mucilage remains sticking as a viscous drop to the tentacles. It is difficult to remove from the tentacle glands in a fresh condition. The quantity of it is large; in the experiment of table 4 it amounts in a fresh leaf to about 57 % of the fresh weight of the leaf without mucilage. After its removal another 19 % mucilage is secreted in the next 24

TABLE 4

Determination of the secretion of 3 series of 6 leaves with tentacles.

Weight fresh weight	Weight after washing	secretion	Weight after 24 hrs. on filter paper	Weight after washing	Newly secreted
373 mg	221 mg	152 mg	265 mg	225 mg	40 mg
368 „	236 „	132 „	287 „	242 „	45 „
344 „	234 „	110 „	284 „	236 „	48 „
1085 mg (157 %)	691 mg (100 %)	394 mg (57 %)	836 mg (121 %)	703 mg (102 %)	133 mg (19 %)

hours. Therefore the leaves with the tentacles were washed in tap-water at the end of the experiment in order to remove the mucilage altogether. Next they were dried on the outside with filter paper and the fresh weight was determined. It is evident that while being washed the leaves may take up water, which influences the fresh weight. A number of weighing tests proved that this procedure gives usable results in many cases.

Reference value. As a reference value for the uptake of transport substance the fresh weight was used in most experiments, the absolute value of the absorbed substance per series of six leaves being determined at the same time. By expressing the uptake per 1000 parts fresh weight the errors are not objectionably great in the kind of experiments treated in this publication. In another kind of experiments, such as those with permeating substances, difficulties by washing arise which will not be discussed here. The blank series has always been treated perfectly analogically to the experimental series and continued in contact with the agar until the end of the experiment, the only difference being that the transport substance had been omitted from the substances added to the agar. This is necessary, because the whole treatment affects the final fresh weight.

Agar. To the use of agar-agar as a medium for adding the substances to be absorbed there are various objections. Agar contains various substances. To be sure it is possible by digesting and electrodialysing to obtain a purer agar, but experience teaches that it is often less fit for use and that it is especially desirable to use a uniform product, though it may contain some nitrogen and phosphate. In the experiments it is a question of difference in uptake between control- and experimental series. Especially in the later experiments we have used commercial products such as Difco agar and Pectacon. In control experiments it appeared that from this agar no nitrogen or phosphate in analysable quantity was absorbed. It should, however, be taken into account that the agar does contain substances which are taken up by the tentacles and cause aggregation. Experiments with digested agar, in which evidently decomposition products were formed from the proteins, often showed highly aggregated tentacles.

The leaves with the tentacles between the agar strips are put in closed Beyerinck boxes in which the atmosphere is kept humid. The experiments were made in a room of constant temperature at 25°. Absorption always took place in the dark.

Analysis. The presence of nitrogen was shown by the Micro-Kjeldahlmethod according to Pregl. Usually total nitrogen was determined after destruction by the salicilic acid method. Phosphate was determined with ammoniummolybdate after destruction of the leaves with concentrated sulphuric acid. The phospormolybdenic acid formed, is reduced with metol and sulphite of soda to a blue colour, which with the aid of a Cenco photelometer is compared with standard phosphate solutions treated in the same way.

For experiments under anaerobic conditions an anaerobic jar of Mc Intosh and Fildes was used. First purified nitrogen gas is passed through next the oxygen still present is combined with added hydrogen gas with the aid of a palladium catalyst.

To Mrs H. Knobbe-Meester I am greatly indebted for the careful execution and help with the experiments and the analyses in the years 1946 to 1952.

II. Active and activated absorption

Drosera tentacles are organs capable of transporting a number of substances from the insects caught to the leaf blade. Besides a secretory function they bring by their curving the adhering insects in touch with a great number of tentacles, and subsequently they also have a transport function (Darwin). Our reason for these researches has been the assumption that the transport process in parenchymatous tissue can best be studied in organs specialized for this function. The uptake of asparagine by the tentacles and the transport to the leaf was first quantitatively demonstrated in Oudman's thesis (1936). He found for this process a high temperature-coefficient and inhibition through narcosis. Arisz and Oudman (1937) and Arisz (IV 1944) pointed out a possible relation between active transport and cytological changes in the tentacle cells, known as aggregation. These experiments are still being continued.

Independence of the transport of water and that of substances. Arisz (I 1942) showed that the transport process is not influenced by water currents in the tentacles, caused by transpiration of the leaf blade or withdrawal of water from the tentacles by adding to the agar an osmotic substance. It is for instance possible to add sugar to the agar and by doing so to withdraw water from the tentacles osmotically. Water and substance transport are therefore independent processes. Water transport mainly occurs in the spiral vessel of the tentacles, the substance transport in the parenchyma.

Diffusion. In the case of some substances as caffeine, we get the impression that they diffuse from cell to cell in the tentacles; this process can be studied under the microscope (Kok 1933). The caffeine permeates into the vacuoles and causes there granulation, which may be used as an indicator for the rate of the transport. From the microscopic findings it appears that diffusion takes place in rows of cells. From this it may be concluded that not the longitudinal wall, but the vacuole is the transport track, while diffusion must take place from vacuole to vacuole through the transverse wall and the adjoining plasm. Yet the transport of caffeine is not purely a diffusion process, because in an anaerobic medium its progress is slower than in an aerobic one (Arisz II 1942). This indicates an influence of metabolic processes in the plasm.

Activating the transport by oxygen. For a great number of substances it has been traced whether they are taken up by the tentacles and transported and whether these processes are influenced by withdrawal of oxygen. A separation of uptake- and transport processes is impossible. When transport is mentioned, we must consider that the uptake may be repeated in every cell, while uptake always implies transport.

It has appeared that the uptake and the transport of all substances which are carried by the tentacles, are to a certain degree dependent on oxygen, so that some substances are transported anaerobically fairly equally, some less strongly or even not at all. This dependence on oxygen we have called activation and as a measure has been taken

the difference of the total aerobic transport from the transport in anaerobic conditions as a percentage of the total transport.

$$\text{Activation} = \frac{\text{transport dependent on oxygen}}{\text{total aerobic transport}} 100.$$

Table 5 gives a survey of the activation for the transport of a number of substances. The data previously published (ARISZ II tables 1 and 2) mainly correspond with this. Deviations are due to the greater number of observations now available and especially to

TABLE 5

Dependence of the transport on oxygen.

$$\text{activation} = \frac{\text{total aerobic transport-anaerobic transport}}{\text{total aerobic transport}} 100$$

	Activation
amino acids	± 100 %
asparagine and glutamine	
NH_4 (from NH_4Cl)	± 100 %
phosphate (from KH_2PO_4)	± 100 %
urea	± 76 %
thiourea	± 52 %
caffeine	± 20 %
ammonium carbonate	± 20 %

the use of lower concentrations of the substances transported. The relative inaccuracy of the experiments does not admit of a more accurate determination. It appears that there isn't a close separation between substances actively taken up and substances penetrating through diffusion, but that there exists a gradual transition.

The transport of amino acids, asparagine and glutamine, likewise of phosphate and ammonium is 100 % activated in sufficiently low concentrations. In higher concentrations some transport also takes place in an anaerobic medium. In the series urea, thiourea, caffeine and ammonium carbonate, activation decreases and in the same order these substances permeate better into the vacuole. This is in accordance with the rule previously mentioned (ARISZ 1942) that the transport is more strongly activated according as the substances permeate less well through the plasm.

By permeation we understand the passive diffusion of substances into or through the protoplasm. If absorption takes place through processes connected with metabolism, we do not speak of permeation, but of active absorption or secretion.

Active transport. For those substances for which the activation amounts to about 100 % we speak of active transport. Active transport is only possible for substances which can hardly or not at all permeate into the cell through diffusion. Dissocia ted substances, such as amino acids and salts on the whole penetrate the boundary surfaces of the protoplasm with difficulty, as is known from plasmo lysis experiments. Once taken up by

TABLE 6

Absorption of *glycine* by the tentacles at 25° C, in darkness. 0.35 M sucrose has been added to the agar for keeping quiet the tentacles

Conc. of glycine in mM in the agar	Absorbed nitrogen per 1000 parts fresh weight during 24 hrs	Conc. of glycine in mM in the leaves	Accumulation factor
50	0.99	70.7	1
25	1.11	79.3	3
12.5	1.04	74.3	6
6.25	0.96	68.6	11
3.125	0.84	60.0	19
	during 42 hours		
3.125	1.96	140.0	45
0.781	0.73	52.2	67
0.195	0.37	26.4	135

Absorption of *asparagine* by the tentacles at 25° C in darkness during 24 hours.

Conc. of asparagine in mM in the agar	Absorbed nitrogen per 1000 parts of fresh weight	Conc. of asparagine in mM in the leaves	Accumulation factor
50	1.47	52.5	1
12.5	1.90	67.9	5
3.125	1.50	53.6	17
0.781	0.80	28.6	37
0.195	0.20	7.1	37

Absorption of *phosphate* by the tentacles in 24 hours at 25° C

Conc. of KH_2PO_4 in mM in the agar	Absorbed P_2O_5 per 1000 parts of fresh weight	Conc. of KH_2PO_4 in mM in the leaves	Accumulation factor
50	0.71	10	0.2
12.5	0.71	10	0.8
3.125	0.72	10	3.2
0.781	0.66	9.3	11.9
0.195	0.41	5.8	29.6

Absorption of *caffeine* by the tentacles in 24 hours at 25° C

Conc. of caffeine in mM in the agar	Absorbed N per 1000 parts of fresh weight	Conc. of caffeine in mM in the leaves	Accumulation factor
50	0.81	14.5	0.29
12.5	0.38	6.8	0.54
3.125	0.17	3.0	0.96
0.781	0.—	—	—

the tentacles, they are no more secreted to the medium. Substances' the transport of which is due to a certain degree to passive permeation and diffusion, may be given off again, if the tentacles are put in water.

Aggregation. Aggregation of the cells, by which the vacuoles are strongly dehydrated and break up in small pieces of a different shape, water being secreted to the plasm, is especially met with substances with highly activated transport. The cytological picture indicates that the active transport must take place in the plasm. Some substances with weaker activation, as urea and ammonium carbonate likewise cause aggregation. The latter substance, however, only in an extremely diluted concentration.

Besides in the behaviour during exosmosis and in the dependence on oxygen the difference between active and activated transport also appears from the relation between uptake and medium concentration. With actively transported substances already from a low concentration a relatively great quantity of substance is absorbed (table 6). Saturation is attained for glycine and asparagine at about 1/80 M, for phosphate already at 0.003 M. For non-actively transported substances, as caffeine the uptake diminishes much more on decrease of concentration. The uptake in the time takes place at a fairly constant rate (see fig. 1. p. 238. Arisz III 1944).

Transport to the leaf. The substances are taken up by the glands at the end of the tentacles and transported to the leaf by the about 4 mm long marginal tentacles. The tentacles don't seem to take up anything at their lateral walls, the pedicel being surrounded by badly permeable outer cell walls. On the sides of the pedicel one or more simple glands may occur through which certain substances penetrate (Coelingh). This, for instance, obtains for caffeine, ammonium carbonate, methylene blue and others. Substances absorbed by the tentacles in 24 hours or longer are for the greater part transported to the leaf. In the tentacles little of the transport substance remains. Naturally this depends on the nature of the transport substance. Caffeine and ammonium carbonate give a clearly visible deposit (granulation) in the vacuoles. In quantitative determinations this quantity proves in most cases to be hardly significant (table 7).

It has not been investigated whether the absorbed substances are

TABLE 7

Uptake of 1/20 M $(NH_4)_2 CO_3$ during 24 hours

	N in γ	N increase in γ	Absorbed N per 1000 parts of fresh weight
before the uptake	724		
after the uptake			
in intact leaves	1364	640	2.60
in leaves without tentacles. . . .	1218	494	2.49
in the tentacles	67		

transported as such or after conversion and how they are deposited in the leaf. Formation of protein from the absorbed amino acids or asparagine OUDMAN (1936) could not demonstrate. If one calculates the concentration of the absorbed substance per fresh weight of the leaf, one finds an accumulation factor, which, for instance, for 0,0002 m asparagine amounts to 37 after an uptake of 24 hours (ARISZ I), for 0,0002 m glycine after 42 hours 135 (ARISZ III), for 0,0002 m KH_2PO_4 after 24 hours 30 (ARISZ II). For activated transport lower values are found, for instance for caffeine 0.003 m (ARISZ II) the accumulation factor is 1. This indicates that active transport takes place independent of the fall in concentration medium-leaf, but an investigation to what extent the absorbed substances are converted, is necessary for a clear insight.

Selectivity of the transport mechanism. Active transport is very selective, i.e. only certain substances are taken up by the tentacles and transported to the leaf. They are either polar substances or electrolytes. Only a few saltions are taken up, such as phosphate and ammonium. Whether ammonium is taken up as an ion or as a molecule is still uncertain. For the ions and the amphoions of the amino acids the transport mechanism must have a specific affinity (ARISZ III). It is possible that the active mechanism is not specifically directed to potassium ions, but that they are absorbed simultaneously with the actively absorbed anions.

III. SIMULTANEOUS ABSORPTION

An insight into the specificity of the absorption mechanism may be obtained by having various substances or ions taken up simultaneously and investigating whether these processes progress independently, so that summation occurs or that they use the same transport mechanism and consequently influence one another (ARISZ IV).

The specific difference between asparagine and phosphate transport appears from experiments with simultaneous transport. Both substances are transported actively. For each of them the strength depends on the concentration of the substance in the medium, saturation already setting in at a low concentration. If these substances are presented in concentrations higher than the saturation value the absorption summates, so that each substance is taken up to its saturation level (table 8). This indicates that the uptake of phosphate is based on a different process from that of asparagine. Just like asparagine various amino acids behave on simultaneous absorption with phosphate.

Combining two substances of a same type (table 9) has as a result, that from the two together no more can be taken up than the saturation level for this type amounts to. This is for instance the case on simultaneous uptake of asparagine and glycine or of alanine and glycine (ARISZ 1944 IV, tables 1 and 2). Also on combination of NaH_2PO_4 and KH_2PO_4 these phosphates proved not to be absorbed independently. In taking up the plasm does not make any difference between

sodium and potassium salts which is to be expected when the anion is taken up actively. These data show that the tentacles do not distinguish between substances of a similar type, but they do distinguish between amino acids and phosphates. The active absorption, therefore, must be connected with binding to components of the

TABLE 8

Simultaneous absorption of asparagine and KH_2PO_4 during 24 hours. 0.35 M sucrose is added to the agar. (From ARISZ IV)

Agar	Increase of phosphate as P_2O_5 per 1000 parts of fresh weight	Increase of nitrogen per 1000 parts of fresh weight
1/20 M KH_2PO_4	0.39	
1/20 M asparagine		1.21
1/20 M KH_2PO_4 + 1/20 M asparagine	0.31	1.18

TABLE 9

Simultaneous absorption of glycine and alanine. A 48 hours B 24 hours; only in B 0.35 M sucrose is added to the agar

Agar	Increase of N per 1000 parts of fresh weight	
	A	B
1/20 M glycine	1.16	0.91
1/20 M alanine	1.27	0.74
1/20 M glycine + 1/20 M alanine	1.10	0.99

plasm. ARISZ (III 1944) thinks that for amino acids and asparagine it is the amphoions, for salts the an- or kations which are bound to the plasm during the uptake. This theory of binding to the plasm explains the specific absorption of different substances.

A special case was noted on the simultaneous absorption of amino acids, asparagine or phosphates with substances as caffeine, ammonium carbonate and antipyrine. (ARISZ IV 1944 tables 6–12). Tables 1

TABLE 10

Simultaneous absorption of KH_2PO_4 and $(NH_4)_2CO_3$ during 46 hours. 0.35 M sucrose is added to the agar

Agar	Increase of phosphate as P_2O_5 per 1000 parts of fresh weight
1/80 M KH_2PO_4	0.85
1/80 M „ + 1/80 M $(NH_4)_2CO_3$	0.68
1/80 M „ + 1/40 M „	0.38
1/80 M „ + 1/10 M „	0.04

and 10 demonstrate this phenomenon. The three last mentioned substances penetrate into the tentacles and cause, as has already been discussed, a granulation in the vacuole. In a sufficiently strong concentration they inhibit the active absorption and the active transport (ARISZ IV tables 7–12). They do not inhibit through an osmotic influence, as sucrose and salts do in a sufficiently high concentration but through an influence on the plasm. In table 1 this has been shown again for caffeine, when through a higher caffeine concentration in spite of the fact that sucrose in lower concentration has been added, absorption is greatly inhibited. It is a remarkable fact that these same substances also inhibit the curving of the tentacles (DARWIN, ÅKERMAN) and inhibit the setting in of aggregation or cause an aggregation already set in to decrease. This too points to a relation between active uptake and aggregation (ARISZ IV 1944).

IV. INFLUENCE OF TEMPERATURE

The influence of the temperature on the uptake and transport processes has been investigated extensively. Table 11 gives a survey of the results obtained. The absorption has been given in millimol. The substances have been arranged in accordance with the magnitude of the Q_{20}. For caffeine and phenylurea this is 2,6, for a low concentration of $(NH_4)_2CO_3$ 3: for a high concentration of NH_4Cl 5, for asparagine likewise 5, for urea 9, and for phosphate 18. It is evident that these determinations cannot lay claim to great accuracy, though

TABLE 11

Influence of the temperature on the absorption of different substances. The uptake is given in millimol per 24 hours

	25° C	Q 15°–25°	15° C	Q 5°–15°	5° C	Q 5°–25°
1/20 M caffeine	20	1.6	12.6	1.6	7.6	2.6
1/20 M phenylurea . . .	22.8	1.4	16.–	1.8	8.6	2.6
1/80 M $(NH_4)_2CO_3$ . . .	77.–	1.3	57.5	2.2	25.4	3.
1/20 M NH_4Cl	56.4	1.5	36.4	3.4	10.7	5.–
1/100 M asparagine . . .	22.7	1.6	14.6	3.4	4.3	5.–
1/20 M urea	28.5	1.5	19.6	6.5	3.–	9.
1/200 M KH_2PO_4	3.6	1.5	2.3	11.–	0.2	18.–

they are averages from a great number of observations. The uptake at 5° C lies for most substances hardly beyond the limits of error. It is a striking fact that the Q 10 for the temperature interval 15–25° C is fairly equal for all substances and rather low, 1.5; whereas greater differences occur for the interval 5–15° C. Here the temperature coefficient is distinctly higher for a more activated transport.

This gives rise to the supposition that the temperature coefficient for active transport is higher than for the weakly activated transport but that between 15° and 25° it does not show, because another factor becomes limiting.

V. Influence of inhibitors

As it had appeared from the dependence of transport on oxygen concentration in the medium, that there must be a relation between transport and metabolism, it seemed expedient to investigate the influence of inhibitors of respiration enzymes in the transport process. These are added to the agar containing the transport substance, in consequence of which they can exercise an influence on the uptake by the gland of the tentacle. Apparently the influence of these inhibitors is *local*. Whether they can be absorbed themselves and transported to other places in the tentacles is uncertain. Sometimes in stronger concentrations they do have an influence on the phenomenon of aggregation, as dinitrophenol and jodoacetate. This is in favour of the supposition that such an inhibitor penetrates. In experiments with oxygen withdrawal the whole tentacle together with the leaf have to be put in surroundings free from oxygen. So here the effect is not localised and it is uncertain whether the processes inhibited by oxygen withdrawal are to be found in the tentacles or in the leaf or in both. This localisation of the active processes can be traced by inhibitors. Besides it is possible to investigate the influence of inhibitors on the transport in tentacles without glands and to decide in this way whether a transport in tentacles without glands is still sensitive to inhibitors. We will first discuss the transport in intact tentacles.

Tentacles with glands. The following inhibitors were examined: KCN, Na-azide, jodoacetate, NaF, Na-arsenate, Na-arsenite and 2-4, dinitrophenol. In addition penicillin, phloridzin, are involved in this research. In concentrations lying between 10^{-2}–10^{-7} mol the inhibitors were added to the agar containing the transport substance. The pH was raised to 6 by means of a Beckman pH meter with glass electrode. Taking into consideration the great variability of the determinations already discussed, which is a result of difference in age of the leaves and the plants and of the season, it is impossible to give graphs in order to compare the inhibitory action of different concentrations of the inhibitors. We restrict ourselves, therefore, to communicating the results of a number of experiments. In one experiment 5 concentrations could be compared. This gave a clear result. But experiments made in different seasons of the year gave results which, though quantitatively noticeably different, did correspond qualitatively. We, therefore, restrict ourselves to giving the limits within which in various experiments 50 % inhibition of the uptake was found. Moreover a choice has been made from the great number of experiments made. In the tables some characteristic inhibitions have been inserted. As already mentioned on discussing the method, collecting in *one* series 6 leaves of various ages is inconvenient for metabolic researches, because the concentrations working inhibitively, are certainly also connected with the ages of the plants and leaves. We restrict ourselves in this discussion to experiments made with two transport substances, asparagine and phosphate (KH_2PO_4). These have been chosen, because it had appeared from the preceding

investigations that both substances are transported actively, but that the mechanism of the uptake of these substances must be different. Asparagine was examined mostly in a concentration of 1/100 M, potassium phosphate in one of 1/50 or 1/80 M. The uptake usually lasted 24 hours, for phosphate sometimes 48 hours. Table 12 gives the concentration of the inhibitors giving 50 % inhibition. In table 13 the results of some experiments are collected. KCN, NaN_3, Na-arsenite, jodoacetate are all substances known as inhibitors of processes forming part of respiration and glycolysis. From the figures

TABLE 12

Average conc. of inhibitor which gives a 50 % inhibition of the absorption of 1/80 M KH_2PO_4 and of 1/100 M asparagine during 24 hours

KCN	10^{-4} M
Na-azid	10^{-5} M
Na F	10^{-2} M
Na-arsenite	10^{-5} M
jodoacetate	10^{-5} — 10^{-6} M

TABLE 13

Inhibition of uptake of asparagine and phosphate by KCN, NaN_3, Na-arsenite and jodoacetate

M conc. of KCN	24 hours' uptake of 1/100 M asparagine, N increase in ‰ of fresh weight	24 hours' uptake of 1/20 M asparagine, N increase in ‰ of fresh weight	Uptake of 1/100 M KH_2PO_4, 48 hours increase of P_2O_5 in ‰ of fresh weight
—	1.09	0.80	0.55
10^{-4}	1.06	0.62	0.53
$3.\ 10^{-4}$	0.78	0.34	0.50
10^{-3}	0.20	0.28	0.43
$3.\ 10^{-3}$	0.02	0.18	0.10
M conc. of NaN_3			
—	0.78	0.92	0.74
10^{-6}	0.57	0.77	0.82
10^{-5}	0.32	0.33	0.79
10^{-4}	0.05	0.21	0.51
M conc. of Na-arsenite			1/80 M KH_2PO_4 24 hours
—	0.80	0.94	0.77
10^{-6}	0.57	0.74	0.55
10^{-5}	0.14	0.29	0.37
10^{-4}	0.09	— 0.02	0.27
10^{-3}	0.02	0.02	0.17
M conc. of jodoacetate			1/100 M KH_2PO_4 24 hours
—	0.92	1.69	0.58
10^{-6}	0.82	0.48	0.35
10^{-5}	0.35	0.08	0.25
10^{-4}	— 0.16	0.05	0.16
10^{-3}	— 0.96	— 0.02	0.10

the conclusion may be drawn that inhibition of respiration also causes inhibition of uptake of asparagine and phosphate. None of these substances behaved differently with regard to transport of asparagine or phosphate. Neither is this to be expected, when the two processes are connected with respiration in a similar way. An application of these inhibitors to the gland of the tentacles, therefore, is sufficient to bring about an inhibition of the transport.

Dinitrophenol is regarded by various investigators as a substance which likewise interferes in the respiration system, does not influence oxydation processes, but represses transphosphorylation (Green, Loomis and Lipmann). Under influence of the dinitrophenol the phosphate is freed from the cyclophorase-gel as an inorganic phosphate (Teply). This so called gel-phosphate has proved in certain cases indispensable for the transfer of phosphate to other substances. Hunter (1951) finds the action of dinitrophenol limited to phosphorylations coupled with electron transfer from $DPNH_2$ to oxygen.

It appeared that in Drosera tentacles dinitrophenol inhibits the uptake of both substances (table 14). From this it may be inferred that the uptake of both asparagine and phosphate depends on transphosphorylation processes, in which energy-rich phosphates are

TABLE 14

Inhibition of asparagine and phosphate uptake by 2–4 dinitrophenol

M conc. of dinitrophenol	24 hours' uptake of 1/100 M asparagine, N increase in $^0/_{00}$ of fresh weight	24 hours' uptake of 1/80 M KH_2PO_4 increase of P_2O_5 in $^0/_{00}$ of fresh weight
—	1.06	0.50
10^{-7}	0.74	0.40
10^{-6}	0.19	0.07
10^{-5}	— 0.01	— 0.07

formed. A similar behaviour shows dinitrophenol in other processes of the plant which require energy, such as growth (Thimann and others, Bonner and Bandurski) saltabsorption by the root (Robertson and others) and active water absorption (Hacket and Thimann).

According to Gale (1949) 8-hydroxyquinoline inhibits the uptake of glutamic acid in bacteria. It was also active in Drosera (table 15). It is a substance that combines with heavy metals.

TABLE 15

Inhibition by 8-hydroxy-quinoline

M conc. of hydroxy-quinoline	24 hours uptake of 1/100 M asparagine, N increase in $^0/_{00}$ of fresh weight	48 hours uptake of 1/100 M KH_2PO_4, increase of P_2O_5 in $^0/_{00}$ of fresh weight
—	0.78	0.43
10^{-4}	0.82	0.46
$3.\ 10^{-4}$	0.81	0.45
10^{-3}	0.66	0.27
$3.\ 10^{-3}$	0.18	0.14

A special discussion requires the behaviour of the remaining substances examined, phloridzin, penicillin and Na-arsenate. These substances proved to work more specifically than the ones already mentioned, because they inhibited the transport of one definite substance either of asparagine or of phosphate. Phloridzin inhibits the transport of phosphate, whereas penicillin and Na-arsenate, only inhibit the aparagine transport. Of the many experiments made with these substances, we have collected some in the table subjoined (table 16). It is known that some preparations of phloridzin have no good effect, unless they have first been purified (STREET and LOWE). The

TABLE 16
Inhibition by phloridzin

M conc. of phloridzin	24 hours' uptake of 1/100 M asparagine, N increase in ‰ of fresh weight	24 hours 'uptake of 1/80 M KH_2PO_4 increase of, P_2O_5 in ‰ of fresh weight
—	0.76	0.60
$2.\ 10^{-6}$	0.80	0.45
$2.\ 10^{-5}$	0.79	0.32
$2.\ 10^{-4}$	0.75	0.27
$2.\ 10^{-3}$	0.71	0.19

preparation we used first (MERCK) also gave a proper inhibition without purification. The preparation of the British Drug H. gave inhibition after purification. It has not been investigated to what extent the addition of sucrose or KCl to the agar to keep the tentacles quiet, influences inhibitive action. Phloridzin was the only substance with which a specific inhibition of the phosphate absorption was obtained. It is known that in a high concentration phloridzin inhibits the phosphorylation of glucose. According to Shapiro it already inhibits in a low concentration the formation of energy-rich phosphates, which is coupled with pyruvate and citrate oxidation.

Penicillin had been used by us as an antibioticum to promote the sterility of our experiments. It then appeared, however, that it had an inhibitive effect on the transport of asparagine; in the phosphate transport no inhibition occurs (table 17). By GALE and TAYLOR, GALE and RODWELL the inhibition by penicillin of the uptake of amino acids especially of glutamic acid has been examined for "growing" cells of *Staphylococcus aureus*. GALE (1949) ascribes the action of penicillin to a disorganisation of the metabolism of ribonucleic acid. GROS and MACHEBOEF (1949) were of opinion that the consumption or the synthesis of mononucleotides are factors in the action of penicillin, other investigators (HOTCHKISS) think that penicillin affects the synthesis of proteins from amino acids. It is evident that this problem has not yet been solved (BROWNLEE 1951). At any rate it is noteworthy that in Gram positive bacteria and in tentacles of Drosera the uptake of amino acids, asparagine and glutamine is inhibited by penicillin. The fact that penicillin does not inhibit the uptake of phosphate and does inhibit the uptake of asparagine even if phosphate

has been added, is in favour of a specific inhibition of the binding of asparagine to a plasm component. An analogous inhibition of penicillin we found for *Vallisneria* leaves in which the uptake of chlorine and phosphate is not inhibited, but the uptake of asparagine is.

TABLE 17
Inhibition by penicillin

conc. of penicillin	24 hours' uptake of 1/100 M asparagine, N increase in $^0/_{00}$ of fresh weight	48 hours' uptake of 1/200 M KH_2PO_4, increase of P_2O_5 in $^0/_{00}$ of fresh weight
—	1.35	0.75
1 O.U./ml	1.16	0.75
2 „	0.74	0.77
4 „	0.58	0.76
conc. of penicillin	24 hours' uptake of 1/100 M glutamine, N increase in $^0/_{00}$ of fresh weight	24 hours' uptake of 1/100 M asparagine, N increase in $^0/_{00}$ of fresh weight
—	1.00	0.77
2 O.U./ml	1.05	0.83
3 „	0.92	0.70
4 „	0.55	0.19
8 „	0.28	0.14

Na-arsenate replaces inorganic phosphate in oxidative systems, in which the uptake of phosphate is coupled to oxydation of the substratum (WARBURG and CHRISTIAN). Addition of phosphate inhibits the action of the arsenate in proportion to the quantities in which these substances are present. BONNER found that arsenate inhibits growth while respiration remains unchanged.

In Drosera tentacles arsenate inhibits the uptake of asparagine, but not the uptake of phosphate (table 18). This need not indicate a specific behaviour of arsenate with regard to the uptake of asparagine, because as we saw above addition of phosphate removes the inhibition by arsenate.

TABLE 18
Inhibition by Na-arsenate

M conc. of arsenate	24 hours' uptake of 1/100 M asparagine, N increase in $^0/_{00}$ of fresh weight	1/100 M asp. + 1/80 M KH_2PO_4, N increase in $^0/_{00}$ of fresh weight	24 hours' uptake of 1/50 M KH_2PO_4, increase of P_2O_5 in $^0/_{00}$ of fresh weight
—	0.97	0.48	0.44
10^{-7}	0.74	0.49	0.47
$3.\ 10^{-7}$	0.59	0.49	0.48
10^{-6}	0.40	0.34	0.46

Tentacles without glands. Before discussing the influence of inhibitors on the uptake and the transport in tentacles without glands, we must first find out to what extent transport by tentacles without glands takes place. It has been known for a long time that tentacles without

glands and also cut tentacles absorb substances as caffeine and that this process is not polar (Kok 1933). Of more importance is here the behaviour of phosphate and asparagine, substances which are polarly and actively transported by tentacles with glands. The transport in tentacles without glands of diluted solutions of phosphate is almost

TABLE 19

Influence of NaN_3 on the absorption during 48 hours of 1/50 M KH_2PO_4 by tentacles with and without glands. 3 experiments A, B, C

M conc. of NaN_3	With glands			Without glands		
	A	B	C	A	B	C
—	0.72	0.92	0.54	0.71	0.96	0.51
10^{-4}	0.62			0.60		
$3.\ 10^{-4}$			0.23			0.22
10^{-3}		0.11			0.21	

as strong as in tentacles with glands (table 19). This process is likewise dependent on oxygen. This is clear from table 20.

The transport of asparagine in tentacles without glands was already examined by Oudman. He found that the uptake from a 1/20 M solution amounts to 73 % of the uptake by intact tentacles. On these experi-

TABLE 20

Uptake of phosphate during 48 hours, in air with and without oxygen by tentacles without glands

	aerobic	anaerobic
48 hours 1/50 M KH_2PO_4 + (KCl + $CaSO_4$) . .	0.95	0.07

ments being repeated (Arisz I table 3), this result was corroborated (table 21). Besides it appeared that the transport in tentacles without glands to a certain extent also depends on oxygen, seeing that in an anaerobic medium half or less than half is taken up. This also indicates that transport is probably not 100 % activated in this case. This need

TABLE 21

Uptake of asparagine during 24 hours by tentacles with and without glands. Two experiments A and B. Uptake as increase of N per 1000 parts of fresh weight

	with glands		without glands	
	A	B	A	B
24 hours 1/20 M asparagine + (KCl + $CaSO_4$)	0.97	1.03	0.55	0.77
1/100 M asparagine + (KCl + $CaSO_4$)	0.70	0.52	0.13	0 12

not surprise us, because also in intact tentacles transport is not 100 % activated in these high asparagine concentrations, in contrast to lower concentrations such as 1/100 M. Moreover on our cutting off the gland the pedicel has been cut and as a result the spiral vessel is opened. Already on this ground it may be expected that in such

tentacles diffusion from high concentrations through the spiral vessel may occur. Therefore it would be preferable to make the experiments with inhibitors at low asparagine concentrations. We found, however, that tentacles without glands take up hardly any asparagine from low concentrations such as 1/100 M (table 21). At any rate this uptake is so slight that a quantitative investigation into the influence of inhibitors is next to useless. (table 22b). So there is an essential difference between the transport of phosphate and asparagine in tentacles without glands. Phosphate is transported as well in an active

TABLE 21*a*

Uptake of asparagine during 24 hours in air with and without oxygen by tentacles without glands

	aerobic	anaerobic
24 hours 1/100 M asparagine + (KCl + $CaSO_4$)	0.14	— 0.10

process as in intact tentacles, but the transport of low concentrations of asparagine is considerably weaker. This gives the impression that the gland is necessary to render an active transport through the pedicel possible. This might be connected with a phenomenon found by COELINGH, i.c. that phosphate causes aggregation also in tentacles without glands, whereas it is not caused by asparagine.

We have now arrived at the discussion of the effect of the experiments with different inhibitors on the phosphate transport in tentacles without glands. For a few inhibitors this yielded indistinct results. The results were variable, which was not the case with our experiments with intact tentacles. It is obvious that these variable results are due to the injury inflicted on the tentacles through cutting off the glands. As a rule KCN, Na-azide, Na-arsenite had a strongly inhibitive influence, NaF weaker, but jodoacetate and dinitrophenol did not inhibit in concentrations which had inhibited in intact tentacles (table 22). First (ARISZ 1952) we were inclined to conclude that the latter two inhibitors should inhibit the transport in the gland, but not in the pedicel. For this conclusion, however, there did not seem to exist proper grounds now that we have a greater quantity of material at our disposal. For the present it is safer to consider only the positive inhibiting effect of KCN, Na-azide and Na-arsenite on the transport in the pedicels of the tentacles without glands as proved. Possibly the other inhibitors do not show their effect in a normal concentration as a result of the injury. This is for instance indicated by the fact that dinitrophenol in a higher concentration 10^{-3} does sometimes act inhibitively. An influence of the pH we could not show. Therefore we must leave the question whether certain substances specially inhibit a reaction in the gland, undecided. The positive result obtained that the active phosphate transport in the pedicel is inhibited independently of the presence of the gland is of essential importance,

TABLE 22*a*

Influence of inhibitors on the uptake of 1/50 M KH_2PO_4 during 48 hours by tentacles with and without glands. Uptake as P_2O_5 increase per 1000 parts of fresh weight

	A		B					
	Normal	2.10^{-5} M dinitro-phenol	Normal	2.10^{-5} M dinitro-phenol	Normal	3.10^{-4} M KCN	Normal	10^{-5} M jodoacetate
with glands	0.75	0.10	0.50	0.06	0.77	0.46	0.66	0.06
without glands	0.77	0.73	0.47	0.17	0.86	0.48	0.70	0.71

	A		B	
	Normal	2.10^{-3} M phloridzin	Normal	2.10^{-3} M phloridzin
with glands	0.93	0.39	1.01	0.49
without glands	0.95	0.94	—	0.28

because it indicates that not only in the uptake in the gland, but as well in the tentacle pedicel processes take place which these inhibitors influence.

We have omitted to investigate the influence of carbon monoxide on the transport, because CO brought into the atmosphere will act as well on the tentacles as on the leaf, so that it cannot be proved whether it influences the processes localized in the tentacle.

As discussed above, the active transport of asparagine in tentacles without glands is slight. This slight transport was markedly inhibited by KCN, Na-azide and Na-arsenite (table 22b). Dinitrophenol and jodo acetate do not inhibit here either. Regarding the specific inhibitors phlo-

TABLE 22*b*

Influence of NaN_3 on the uptake of 1/100 M asparagine during 24 hours by tentacles with and without glands

	normal	10^{-4} M azide
with glands	0.80	0.11
without glands	0.18	0.05

ridzin for phosphate transport and penicillin for asparagine transport, it may be observed that with the exception of one experiment, the inhibition by phloridzin did not occur in tentacles without glands. This one exceptional case makes us doubtful about the significance of the absence of the inhibitory effect found by us. Penicillin, however, was always active also in tentacles without glands and inhibited the weak asparagine absorption. Also the stronger asparagine absorption in tentacles without glands at an asparagine concentration of 1/20 mol was distinctly inhibited (table 23). Summarizing we

TABLE 23

Influence of penicillin on the uptake of asparagine by tentacles with and without glands

	normal	10 O.U./ml penicillin
24 hours 1/20 M asparagine with glands	0.92	0.48
24 hours 1/20 M asparagine without glands . . .	0.68	0.39

think we may state that the investigation into inhibitors in tentacles without glands shows that an active transport takes place in the pedicels.

VI. Influence of some dye-stuffs on the transport

Schumacher used eosin to make the sieve tubes in *Pelargonium* unfit for use. He showed that if eosin is put on a leaf in conc. 1 : 25000–100000 this penetrates into the vascular bundle through the parenchyma and makes the sieve tubes unfit for transport. The sieve tubes

die off and after two or three days callus-formation is found near the sieve plates. After dying the sieve tubes are compressed. All other cells continue living. After that the leaves were no more capable of translocating nitrogen compounds. The same phenomenon was found by SCHUMACHER for a great number of other plants. OUDMAN used this method to inhibit the translocation of N-compounds from the leaves of *Drosera*. It seemed interesting to discover whether eosin also influences the transport in the tentacles. Besides the action of fluorescein-potassium was examined. According to SCHUMACHER (1933) fluorescein gives no callusformation in the sieve tubes. It is easy to recognize by the yellowish green fluorescence under a fluorescence microscope. When put on a *Pelargonium* leaf the sieve tubes and companion cells fluorescence after some hours. Therefore it has been used

TABLE 24

Influence of eosin on the uptake of 1/100 M asparagine and of 1/50 M phosphate during 24 hours

Conc. of eosin	Uptake of asparagine, N increase ‰ fresh weight	Uptake of phosphate, P_2O_5 increase ‰ fresh weight
—	1.22	0.55
0.00003 %	1.00	0.12
0.0003 %	0.60	— 0.02
0.003 %	0.—	0.02

by SCHUMACHER as an indicator of the transport. Also the uptake and the transport of fluorescein in parenchymatous tissue has been extensively examined by SCHUMACHER. It is, therefore, very important for us to know whether this dye influences the transport in parenchymatous tissue.

Eosin appeared to be an extremely toxic substance in *Drosera* tentacles too (table 24). The experiments were made in the dark, so that a photodynamic influence of the eosin was avoided as much as possible. In a concentration of 3.10^{-7} M, it causes a strong inhibition of the

TABLE 25

Influence of K-fluorescein on the uptake of 1/100 M asparagine and of 1/80 M phosphate

Conc. of fluorescein	Uptake of asparagine, N increase ‰ fresh weight	Uptake of phosphate, P_2O_5 increase ‰ fresh weight
—	1.03	0.64
0.001 %	0.90	0.51
0.003 %	0.66	0.51
0.01 %	0.45	0.38
0.03 %	0.26	0.11

transport both of the phosphate and of the asparagine uptake. This result indicates that this substance not only in the sieve cells, but also

in the parenchyma cells, has an inhibitory influence on transport processes in the protoplasm.

Fluorescein-potassium, which according to SCHUMACHER is not toxic, influences the plasmatic transport in *Drosera* tentacles as well (table 25). Lethal action is out of the question, as the tentacles keep living. There is, however, a pronounced inhibition of the transport of asparagine and of phosphate. A 50 % inhibition appears, when $2.7\ 10^{-4}$ M fluorescein-potassium is added to the agar in which the transport substance is present. It is unknown what the inhibition by fluorescein is based on. It is known, however, that triphenyl methan dyes to which both eosin and fluorescein belong, inhibit various metabolic processes. As these dyes act on the uptake of phosphate and of asparagine in the same way, it must be assumed that they influence a general process that is essential for the transport. The tentacles absorb the dye strongly in the protoplasm, but in a phase which does not act a part in the protoplasmic streaming. Already before we could corroborate SCHUMACHER's observation that the extension of fluorescein in the plasm is entirely independent of the plasmic streaming. This makes us surmise that the inhibitive action of the normal transport might be connected with a displacement of the transport substance from a protoplasmatic surface. At any rate it appears from the action of fluorescein that it will have to be used with great caution as an indicator for a normal plasmatic transport. Further investigation is necessary.

VII. Influence of inhibitors during the transport of other substances

As discussed the transport of caffeine is different from that of phosphate and amino acids: it is more like a diffusion process, but it is, be it in a slight degree, dependent on oxygen. The nature of the process finds expression in the following features:

1. In the dependence on the concentration of the medium: in a low concentration little caffeine is absorbed, whereas with actively absorbed substances relatively more is taken up from low concentrations.
2. In the dependence on the temperature; for caffeine the temperature coefficient for lower temperatures is lower.
3. In the dependence on oxygen. Without oxygen the transport is but slightly inhibited. The activation of the transport is slight.
4. In the non-polarity of the transport. The gland of the tentacle has no specific influence, while uptake takes place as much by the base as by the tip of a cut off tentacle.
5. In the non-occurrence of aggregation, as with actively transported substances. Caffeine gives rise to granulation (coacervation) in the vacuole.
6. In the transport track. In caffeine transport the vacuole acts an important part. Passing the transverse wall and the adjoining plasm offers a 160 times greater resistance than passing through the vacuole (KOK).

7. In the leaching of the absorbed caffeine on putting the leaves in water. This is in contrast with the retention of the absorbed substances after uptake of asparagine and phosphate.

Taking into account the slight influence of oxygen on the transport of caffeine, it is not to be expected that inhibitors will have a considerable influence on the transport. The differences found are often too slight to conclude to an inhibitory action with certainty. Let it suffice, therefore, to state that KCN, Na-azide and dinitrophenol gave a slight inhibition at higher concentration.

TABLE 26

Influence of inhibitors on the uptake during 24 hours A of caffeine, B of NH_4Cl and C of $(NH_4)_2CO_3$

A

M conc. of KCN	Uptake of 1/20 M caffeine, N increase in ‰ fresh weight
—	1.34
10^{-4}	1.35
3.10^{-4}	1.32
10^{-3}	0.87
3.10^{-3}	0.51

B

M conc. of dinitrophenol	Uptake of 1/80 M NH_4Cl, N increase in ‰ fresh weight
—	0.76
10^{-7}	0.60
10^{-6}	0.27
10^{-5}	0.07
10^{-4}	0.—
M conc. of jodoacetate	
—	1.19
10^{-6}	0.68
10^{-5}	0.68
10^{-4}	0.54
10^{-3}	0.22

C

M conc. of NaN_3	Uptake of 1/80 M $(NH_4)_2CO_3$, N increase in ‰ of fresh weight
—	3.34
10^{-6}	3.37
10^{-5}	3.28
10^{-4}	2.77
10^{-3}	2.66

For the transport of other substances, urea, ammonium chloride and ammonium carbonate the influence of some inhibitors has also been examined (table 26). Ammonium from ammoniumchloride behaves like phosphate. For the others the influence may be compared with that of caffeine. For urea the inhibition is stronger, which was to be expected, the transport being more strongly activated here. Ammonium carbonate was absorbed very strongly (table 11). It is distinctly inhibited by withdrawal of oxygen (table 5), also at low temperature at 5° C. Anaerobically there arises a green precipitate in the cells of the pedicel, which turns into black when exposed to the air. Na-azide 10^{-4} gives a distinct but slight inhibition (table 26).

VIII. Discussion

From the above it has appeared that to making experiments on uptake and transport in leaves of *Drosera capensis* various difficulties are attached. We summarize them below:

1. Growing regularly developed plants in an adequate quantity. For each experiment 36 leaves, divided into 6 series, are used. Each plant has to possess six successive suitable leaves.
2. The long duration of the experiments, in which the uptake depending on the nature of the substance absorbed must last 24 or 48 hours to obtain a sufficiently strong transport. As a rule two or three experiments a week could be made in the most favourable season.
3. The presence of mucilage on the tentacle glands necessitates washing off the leaves before the fresh weight can be determined. In order to avoid injury this washing can only be done after finishing the experiment. Washing results in an alteration of the fresh weight and with some substances there is leaching due to exosmosis.
4. As a reference quantity the fresh weight of the leaves at the end of the experiments after washing has been used. The uptake can also be determined per series of six leaves. This matters for series of which the leaves are sufficiently equal in length and development and for which owing to the nature of the experiments the final fresh weight is unsuitable as a reference value.
5. The lack of sterility of plants and medium through which organic substances in the medium can be decomposed. The substances have been administered in agar by which we should take into account the impurities which the agar may contain and which may have an influence on the tentacles.
6. Keeping the tentacles quiet, so that they cannot curve from the agar and absorb the substance from the agar unequally. Adding caffeine, sucrose or salts to the agar keeps the tentacles quiet, but at the same time influences the uptake.
7. The composition of a series from 6 leaves of different ages means that leaves that react differently in their metabolism, have been put together in one series.
8. The influence of the season and of external factors during the growth on the reactive power of the plants.

9. The variability in the observations which is due to the many sources of error.

The difficulties mentioned above have been met as well as possible, but some of them are inherent to the material used.

It is a striking fact that with the uptake of all substances an influence of oxygen has been found, which indicates that also with substances that penetrate through diffusion, the influence of the plasm that has to be passed, is expressed in a dependence on respiration. This gives the impression that there is *no strict separation between passively and actively absorbed substances*. In taking up certain substances "physical" and "physiological" permeability, i.e. passive and active uptake can cooperate or in Overton's terminology permeation and adenoid absorption can go together. On the simultaneous action of the two processes we have not yet any data for other objects. Neither has it been taken into account that this possibility exists, so that in researches on permeability active processes are frequently made impossible by the way of experimenting. Injury due to cutting, infiltration to make the preparations more transparent and lack of oxygen will have to be avoided. It is quite possible that if these items are taken into consideration, cases will be met which will correspond with our results with *Drosera*.

Before discussing the results obtained, we must remind the reader of the fact that it is essentially impossible to separate processes of uptake and transport.

In the two preceding communications (Arisz III and IV 1944) we have arrived at a theory to interpret the phenomena found. We will discuss this theory first and next consider to what extent the results newly obtained correspond with it.

Of the active absorption of substances as asparagine, amino acids and phosphates the following presentation was given in 1944(Arisz III. p. 246).

"The first phase of active absorption is based on a combining of ions from the medium with components of the protoplasm in the boundary surface. In the case of amino acids and asparagine it is the amphoions, in the case of the salts the anions which combine with the plasm. According as the concentration in the medium is higher, more ions are combined, but in low concentrations, as is the rule in adsorption processes relatively more ions are combined than in strong ones. On further increase of the concentration the number of particles which can be combined at the same time reaches a marginal value. This marginal value fixes a limit to the loading of the transport system. In this connection we leave the question open of what nature the linkage is between the parts of the substance transported and the protoplasmic carriers. The quantitative relation between rate of absorption and concentration in the medium indicates an adsorption process in which at the boundary surface a quick adjustment of equilibrium takes place, yet we can also think of chemical combinations.

On the first phase of the absorption or transport process follows the introduction of the particles of the transport substance into the cyto-

plasm, in consequence of which fresh particles can be combined at the boundary surface. The view that in order to be absorbed the substances are combined with protoplasmic carriers and do not permeate into the cell through free diffusion we call *the theory of plasmatic binding.*

From the subsequent publication (ARISZ 1944 IV) we quote as follows: If when absorbing the cell does not make any difference between one amino acid and another or between amino acid and asparagine, this means that the protoplasmic patches which can combine with asparagine, can also combine with amino acids. The number of patches, therefore, available for combining with an amino acid or an asparagine amphoion, is limited and these substances compete for a place on the boundary surface. Phosphates are absorbed independently of amino acids and asparagine. This means that the patches capable of combining phosphates, cannot combine with the amphoions of amino acids at the same time. There are, therefore, patches of a different type in the protoplasm, some of which are adapted for combining with amino acids and asparagine and others for combining with phosphate. Of what nature the combining with phosphates is, has not yet been cleared up. It does follow from the experiments that it does not make any difference whether potassium or sodium phosphate is present" (l.c. p. 257).

We now arrive at the question what supplement and alteration is necessary of the standpoint taken up by us in 1944. For this purpose we summarize the data newly obtained on the influence of inhibitors on the active transport. On the whole it may be said that the chemical nature of combining the substances actively absorbed in the protoplasm has become more prominent.

1. The result obtained before that the absorption of asparagine and of phosphate is dependent on oxygen only indicates the connection of the transport with metabolism, but does not show where the active processes are localized. For the analysis of these processes, therefore, inhibitors are of great importance, as they can be administered locally and render conclusions on the localization of the active processes possible.

2. Inhibitors of the respiration such as KCN, Na-azide and Na-arsenite inhibit the transport through the intact tentacles and likewise through the pedicels after the glands of the tentacles have been removed. This indicates that the dependence of active transport on respiration is not limited to a process that takes place in the boundary surface between gland and medium. No more can the active transport be exclusively due to processes that are restricted to the leaf and for instance keep the concentration of the transport substance there at a low level, so that a continuous diffusion from the medium with high concentration through the tentacles to the leaf would become possible. *The cellular transport in the whole tentacle must depend on the respiration.*

3. The deviating behaviour of iodo acetate and dinitrophenol with regard to tentacles without glands could be interpreted in two ways. It may be that as a result of the wounding the inhibitors are inactivated and less active. This is indicated by the fact that in a

higher concentration of dinitrophenol sometimes a strong inhibition in the tentacles without glands is obtained. A second possibility is that these inhibitors are only active in the gland. This is possible, but it does not seem probable, seeing the varying results, in which in a few cases an inhibition is occasionally found.

4. Na-arsenate inhibits the uptake of asparagine, as a result of its inhibiting phosphorylation, so that no phosphates rich in energy are made available. It has, therefore, a similar effect as dinitrophenol. Na-arsenate, however, does not influence the uptake of phosphate. This different effect may be explained satisfactorily by assuming that this inhibitor is displaced from the substratum by inorganic phosphate which is administered to the medium. Inhibition of asparagine absorption by Na-arsenate therefore, is not a specific process attuned to the uptake of one special substance. This is an interesting indication that one should be cautious in ascribing specificity to a particular inhibitor. This was a reason to consider for the other inhibitors working specifically on asparagine whether addition of inorganic phosphate to the medium influences inhibition.

5. Inhibition of phloridzin, and penicillin seems to be specific. Phloridzin inhibits the uptake of phosphate only, penicillin, only the uptake of asparagine (also that of glutamine, while amino acids have not yet been examined). This result corresponds with what is known about the inhibition of these substances in literature (Gale, Nance). The biochemical insight, however, into the reactions which these substances affect, is still insufficient.

The result of these experiments is, therefore, a corroboration of the connection between transport and respiration. Inhibition of the respiration renders transport impossible. It is, however, not clear yet what parts of the respiration process are influenced by special inhibitors. In biochemical litterature it is stated that the uncoupling of the formation of energy-rich phosphates from respiration is caused by a great many substances. But they can effect this in quite different ways. Among these substances are 2,4-dinitrophenol, p. nitrophenol, methylene blue, brilliant cresyl blue, arsenite, arsenate, Ca^{++} and malonate (Lehninger 1951). Spiegelman, Kamen and Sussman 1948 and Loomis and Lipmann 1949 found that azide likewise inhibits the formation of energy-rich phosphates (Spiegelman 1952).

About the way in which these inhibitors act in our experiments with *Drosera* there prevails an uncertainty. Suffice it to state the general connection with respiration and particularly the dependence of the transport processes on the production of energy rich phosphates. In accordance with the previously shown specificity of the uptake of asparagine and phosphate it was found that there are inhibitors which inhibit the uptake of a special transport substance specifically. Penicillin acts on the combining of asparagine and phloridzin on the combining of phosphate.

The theory of transport through plasmatic binding therefore, has been vigorously supported by the experiments with inhibitors. The experience that many inhibitors are active as well in tentacles with-

out glands as with glands indicates that the active part of the absorption and transport process is not limited to the gland, but that in every tentacle cell the same reactions take place and the transport through the tentacle must, therefore, be considered a succession of reactions in the parenchyma cells of gland and pedicel. What part the leaf acts in this remains unexplained, but there is no reason to expect that the transport processes will be different there.

This resolves the problem of uptake and transport by the tentacles into the more general problem of the polar transport of substance in parenchymatous tissue, as is for instance known for growth substances. This problem will not be further discussed here.

About the question whether substances taken up in the plasm of a cell through a metabolic process, are set free there in their original or in changed form or continue combined with plasm carriers, we have not yet got any data. Of great importance for the cellular transport are the newer data on the significance of microsomes and mitochondria as enzyme systems (MILLERD e.a. on *Phaseolus*). We may refer to recent summaries (ARISZ 1952, ROBERTSON 1951, SPIEGELMAN and SUSSMAN 1952).

The connection of the aggregation processes often discussed in this and in preceding publications with the transport processes can only be mentioned here. It seems as if here a possibility exists to enter deeper into the nature of the cellular transport process, but various points still require a further investigation before we can give a summarizing opinion about this. Also the remarkable inhibiting influence of fluorescein on the transport of actively absorbed substances requires a further investigation. It does not seem impossible that the action of this substance is closely connected with the mechanism of the transport in the protoplasm. The action of eosin and fluorescein is not specific. Both asparagine and phosphate transport are inhibited, as much in tentacles with as without glands. In the case of fluorescein it may be easily noted that it permeates into and accumulates in the plasm. In these cells we also frequently meet contraction of vacuoles. The actions of these dyes on the transport suggest a displacement of the transport substances from active enzyme surfaces.

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Summary

The tentacles of the leaves of *Drosera* have the function of secreting to the leaf substances which are liberated by the disintegration of the insects. An extensive survey has been given of the method of research. The difficulties met have been summed up in VIII pp. 98 and 99 and in the methodical part it has been stated to what extent it has been possible to find a working method giving reliable results.

The principal results of two previous publications, which had been published in Dutch in war time, have been summarized and supplemented with new data.

The tentacles of *Drosera capensis* are genuine transport organs, which themselves retain the transport substance in hardly analysable quantities, but pass it on to the leaf. Important is the dependence on oxygen in the medium. Some substances are not transported in an anaerobic medium others only inhibited. The first set of substances is absorbed from a low concentration and accumulated in the leaf in a changed or unchanged form. We have called this active transport. In the latter case we have spoken of activated transport since a diffusion process acts an important part; pure diffusion, however, was not found for any substance, as withdrawal of oxygen always effects the transport somewhat. This points to influence of processes in the plasm, connected with metabolism. Amino acids, asparagine, glutamine, phosphates and ammonium are transported actively. For urea, thiourea, ammonium carbonate, caffeine, and others activation of the transport occurs in a different degree. The transport of amino acids, asparagine and glutamine on the one side and that of phosphate on the other side have in common that these substances are transported actively, but the nature of the transport differs for these two groups of substances. This specificity of the transport appears from summation experiments. For each substance the uptake depends on the concentration in the medium; already in fairly low concentrations a maximal rate of uptake is reached. Combination of different amino acids or of asparagine with an amino acid gives as long as the concentration is limiting summation until the saturation level for amino acids and asparagine has been reached. This indicates that they are all absorbed by the same system. Combination of an amino acid or asparagine with phosphate, however, gives of each of these substances absorption till saturation is attained. These systems must work independently. This led us in 1944 to draw up the hypothesis that during the transport the substances are combined with the plasm. Chemical reactions between the substances and the plasmatic particles must take place rendering absorption and transport possible. The summation experiments indicate that the nature of the binding of amino acids and asparagine is different from that of phosphates. The theory of the transport through combining the substances with the protoplasm based on these data (Arisz 1944) has been discussed in VIII pp 100 and 101.

Some substances such as caffeine, antipyrine, and ammoniumcarbonate inhibit especially in a higher concentration the active transport of phosphates and amino acids. These substances inhibit aggregation as well.

With the aid of substances inhibiting enzymatic processes, the relation of transport to metabolism has been investigated. Such investigations have, for instance, been made by Thimann and collaborators for the growth, the protoplasmic streaming and the active absorption of water and for instance by Lundegårdh, Machlis and

ROBERTSON for the uptake of salts. The results of our experiments was that the wellknown inhibitors of glycolysis and respiration, KCN, Na-azide, jodoacetate and Na-arsenate all inhibit the transport of phosphate and asparagine. Dinitrophenol likewise inhibits the transport of these two substances, which points to the influence of energy-rich phosphates on the transport process. Na-arsenate inhibits the asparagine transport .This likewise points to the significance of energy-rich phosphates for the transport processes. As phosphate competes with arsenate, it is to be understood that arsenate has no influence on the phosphate transport. More specific is the behaviour of phloridzin and penicillin. The first substance only inhibits the uptake of phosphate, the last only the uptake of asparagine. These specific inhibitions prove that the theory previously formulated that the combination of phosphate with the plasm is of a different nature from that of asparagine and amino acids is correct.

The glands of the tentacles can be cut off with a pair of scissors. Marginal tentacles treated in such a way behave essentially in the same way as tentacles with glands. They transport phosphate as well as tentacles without glands; this transport is likewise dependent on oxygen. The glands of the tentacles are, therefore, not essential to the active transport of phosphates. The transport of asparagine by the tentacles, however, is dependent on the presence of glands. For a low asparagine concentration 1/100 M, the transport is very slight. For a higher concentration, for instance 1/20 M, it may be greater, but the height of the transport in intact tentacles is not reached. The gland, therefore, has a specific influence on the uptake of asparagine. This reminds us of the influence of the gland on aggregation (COELINGH).

Inhibitors of the respiration have partly the same influence on the transport of phosphate in tentacles without glands as with intact tentacles. KCN, Na-azide and Na-arsenite inhibit this transport. This proves that the transport in the tentacle pedicel is of the same nature as the transport in the intact tentacles.

Various inhibitors gave no inhibition or a weaker one in tentacles without glands than in intact tentacles. This phenomenon has not yet been explained. It may be connected with the wounding or point to the fact that certain reactions are localized in the gland.

Eosin is a dye which is used to render transport in the sieve tubes impossible. Eosin gives a complete inhibition of the transport in Drosera tentacles already in a very low concentration.

Fluorescein has been used as an indicator of the transport in parenchyma cells and in sieve tubes. It causes a distinct inhibition of the transport in Drosera tentacles in a low concentration.

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PECTINASE INHIBITORS IN PEARS

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Introduction

During the ripening of fruits, insoluble pectin is converted into the soluble form, which in its turn is broken down to galacturonic acid.

Although a pectin splitting enzyme with properties slightly different from pectinase was demonstrated in tomato fruit by McCOLLOCH and KERTESZ (8, 9) and evidence of the presence of similar enzymes in other fruits has recently been put forward by a number of authors, nothing as yet is known with certainty about the causes of the pectin conversions in ripening pears and apples.

In fact, as a result of the failure of many attempts to prove the presence of pectin splitting enzymes in these fruits, KERTESZ suggested that the conversions may well be non-enzymic (7), pointing out that in vitro long-chained pectin molecules could be split into smaller units by ascorbic acid and peroxides (6, 4). However, according to the results of the investigation presented below, the situation is shown to be more complex.

At least two pectinase inhibiting substances were found to be present in the sap of pears. One of these proved to be thermostable and was found only in the early stages of development of the fruit, whereas the other is thermolabile and appears when the fruit is about three months old. The amount then increases and reaches a maximum in the ripe fruit.

There is little evidence in the literature on naturally occurring inhibitors of pectinase. CHONA (2) found that malic acid in apples and potassium phosphate and magnesium sulphate in potatoes inhibited the activity of the enzyme. MEHLITZ and MAASS (10) suggested that tannin also had inhibiting properties, which however, was contradicted by WEBER and collab. (15).

This paper deals only with the thermolabile inhibitor found in *Doyenné Boussoch* and two other varieties of pears. Physiological data and other aspects of the pectin conversions in these fruits will be presented separately.

The older notations "pectinase" and "pectase" for the enzymes are

used instead of polygalacturonase and pectin-methyl esterase to indicate that none of the enzymes used in the experiments have been specially purified.

Methods

One of the most sensitive methods to determine the activity of pectinase is to measure the decrease in viscosity of a pectin solution caused by the enzyme (7); this applies especially to the initial stages of the breakdown.

In our experiments commercial pectinase [1] and different kinds of commercial and self-prepared pectins were used. The viscosity was determined with a Hoeppler falling ball type viscosimeter at 25.00 $\pm$ 0.02° C.

The data obtained in the experiments are presented in the form of graphs. The change in the viscosity of the solutions is expressed as the decrease in the falling time of the ball in the viscosimeter tube and is plotted against the reaction time (Fig. 1).

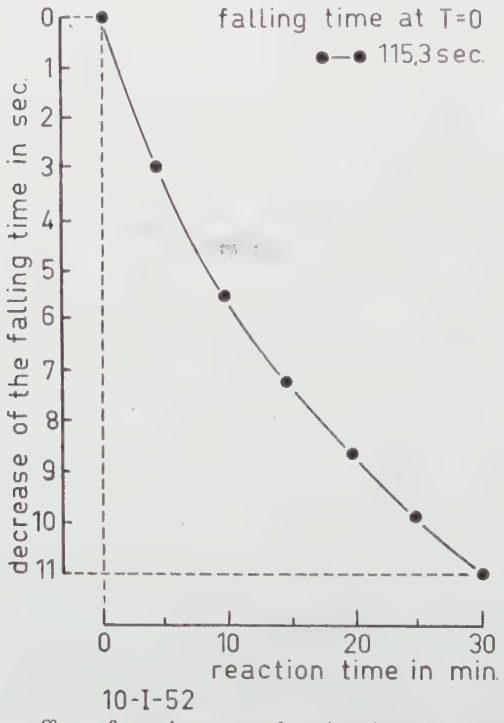

Fig. 1. The effect of pectinase on the viscosity of a pectin solution. 50 ml 0,22 % B.P.-pectin in 0.05 mol. succinic acid-borax buffer, pH 3,6; 1 ml 1 % Pectasin-A. Temp. 25° C.

[1] "Pectasin A" from Polak en Schwartz, N.V., Zaandam. Activity: 30 P.G.U./gr. (20° C); determ. accord. to Kertesz (7). Containing: 3.4 P.M.U./gr. (20° C); determ. accord. to Kertesz (7).

In all figures the falling time at the beginning of the experiment (fall. t. at T = 0) is given and hence the actual falling time during the experiment can be read from the graphs.

Experimental

The presence of a thermolabile pectinase inhibitor in the sap of *Doyenné Boussoch* pears can be demonstrated by comparing the rates of the viscosity drop on the addition of fresh and boiled sap to the reaction mixture respectively (Fig. 2).

Since sap from different varieties of pears or from fruit picked at different stages of development is compared, it should be borne in mind that it contains different amounts of pectin, thus changing the

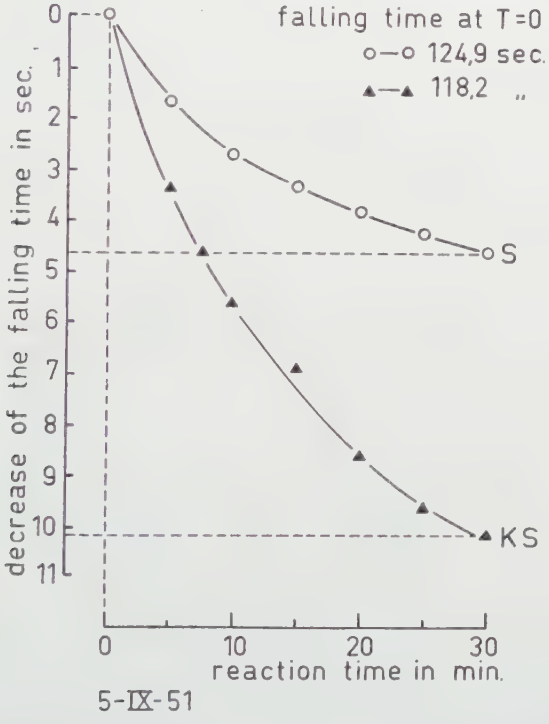

Fig. 2. The effect of the sap of pears on the decrease in viscosity of a pectin solution caused by pectinase.
50 ml 0,19 % B.P-pectin in 0,05 mol. succinic acid-borax buffer, pH 3,6; 5 ml sap (S) or 5 ml boiled sap (KS), 1 ml 1 % Pectasin-A. Temp. 25° C.

initial concentration of the reaction mixture. As a consequence the rate of decrease in viscosity by a certain amount of enzyme will be changed as well. The Figs. 3 and 4 give the extent of this influence in the range of the pectin concentrations used in the following experiments. From these graphs and the falling time at T = 0 given in

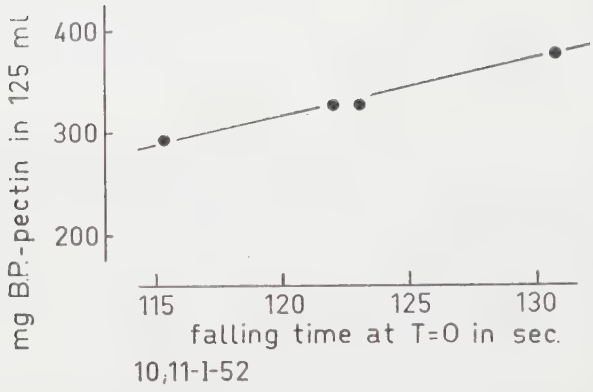

Fig. 3. The relation between the concentration of B.P.-pectin in succinic acid-borax buffer, pH 3,6, and the viscosity.

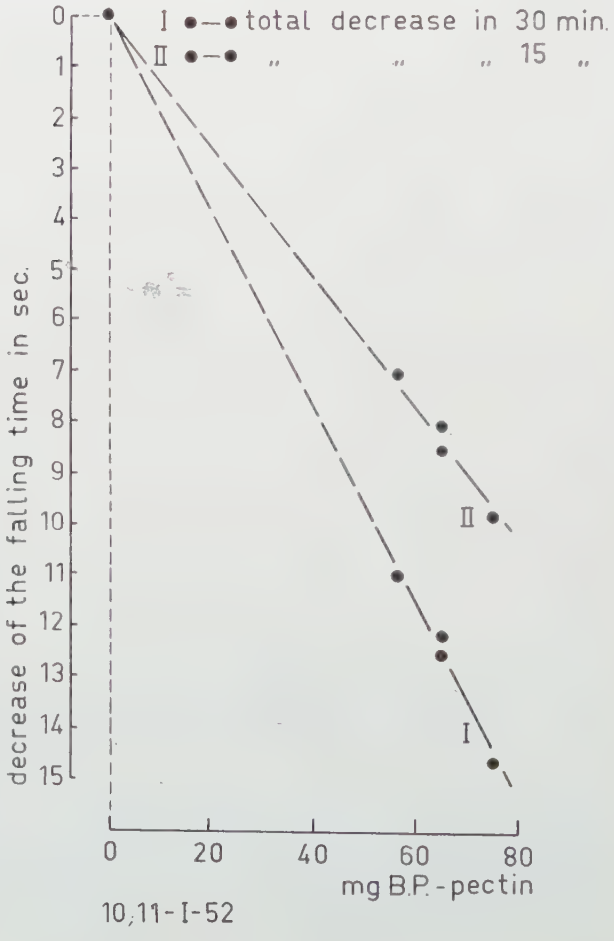

Fig. 4. The relation between the concentration of a pectin solution and the decrease in viscosity caused by pectinase in 15 and 30 min.

50 ml B.P.-pectin in 0,05 mol. succinic acid-borax buffer, pH 3,6, containing the amount of pectin indicated in 25 ml; 1 ml 1 % Pectasin-A Temp. 25° C.

all figures it is clear that the interpretation of the results is not altered by this factor.

However, as a result of the method used, the fact that the decrease in viscosity is lessened by the sap should not be attributed to an inhibitor of the enzyme without considering other possible explanations.

Since the demethoxylating enzyme pectase can increase the viscosity of pectin solutions, the addition of sap containing this enzyme to the reaction mixture might produce effects, which cannot be distinguished from a true inhibition of the pectinase (Fig. 5).

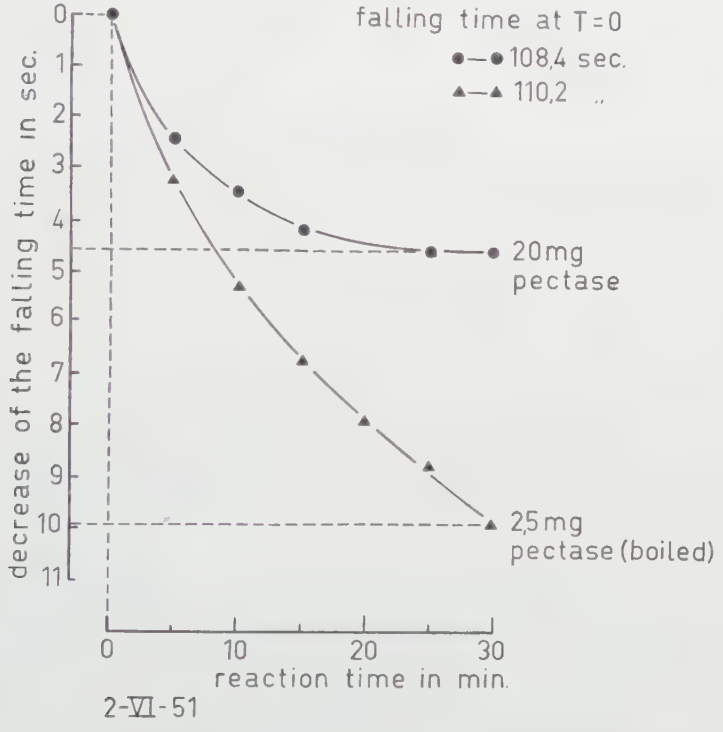

Fig. 5. The effect of pectase on the decrease in viscosity of a pectin solution caused by pectinase.
50 ml 0,19 % B.P.-pectin in 0,05 mol. succinic acid-borax buffer, pH 3,6; 5 ml 0,4 % citrus-pectase; 1 ml 1 % Pectasin-A. Temp. 25° C.

On the other hand, small amounts of pectase present in the pectinase preparation used in the experiments strengthen the activity of the latter enzyme (5, 7), so an inhibition of the pectase might result in a decrease of the pectinase activity.

Other possible explanations for the phenomena observed will be dealt with below.

All the experiments presented below were performed with the inhibitor, RI, isolated from the sap by precipitation with three times the volume of acetone. The precipitate, washed free of water with

acetone and dried by air, showed in aqueous solution the same pectinase inhibiting properties as the sap itself; boiled solutions, having no influence on the pectinase, were used as controls.

In the Figures 6, a–f, experiments are presented which strengthen the likelihood that the substance isolated from the sap is indeed an inhibitor of pectinase. In these experiments pectic acid was used as a substrate for the enzyme instead of pectin. Separate tests showed that the viscosity of the pectic acid solutions was not changed by citrus pectase. By comparing the graphs a and b it is seen that the total decrease in the falling time, caused by the pectinase, is 9.2 and 4.6 sec./30 min. when the inactivated and the active RI solutions are added respectively.

Since the activity of the pectinase is lessened when either pectic acid or pectin is used as a substrate, explanations in which any influence is attributed to pectase or pectase inhibitors are untenable.

Changes in the pH of pectin solutions may change their viscosity

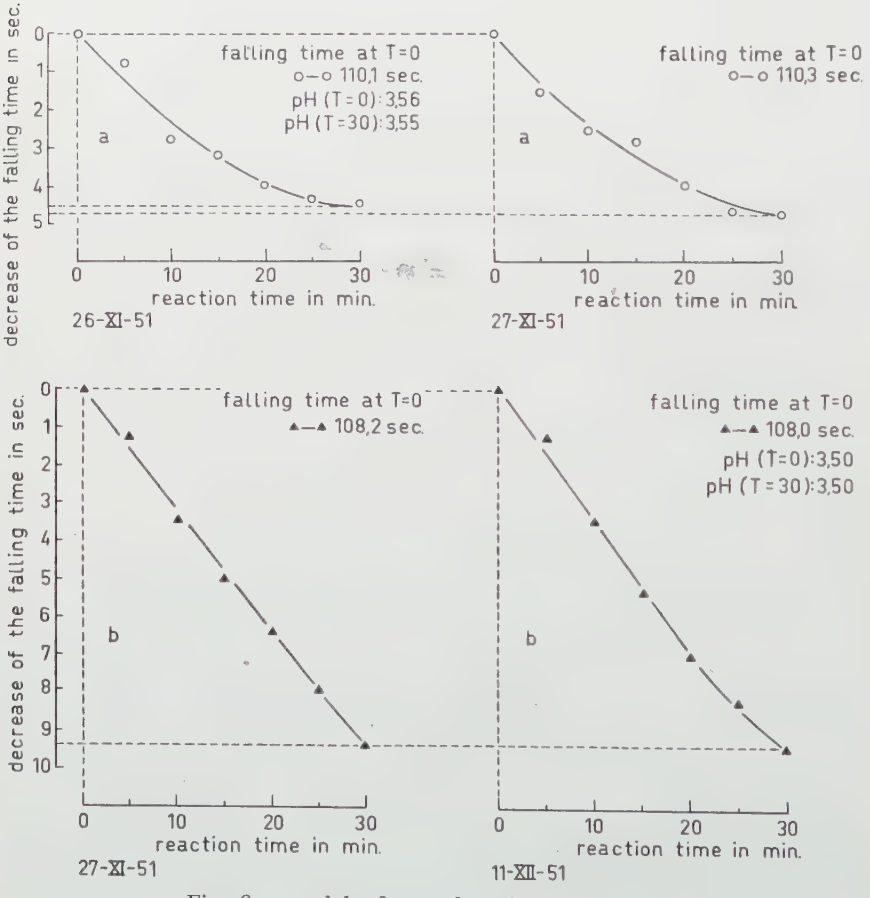

Fig. 6, a and b; for explanation see p. 113

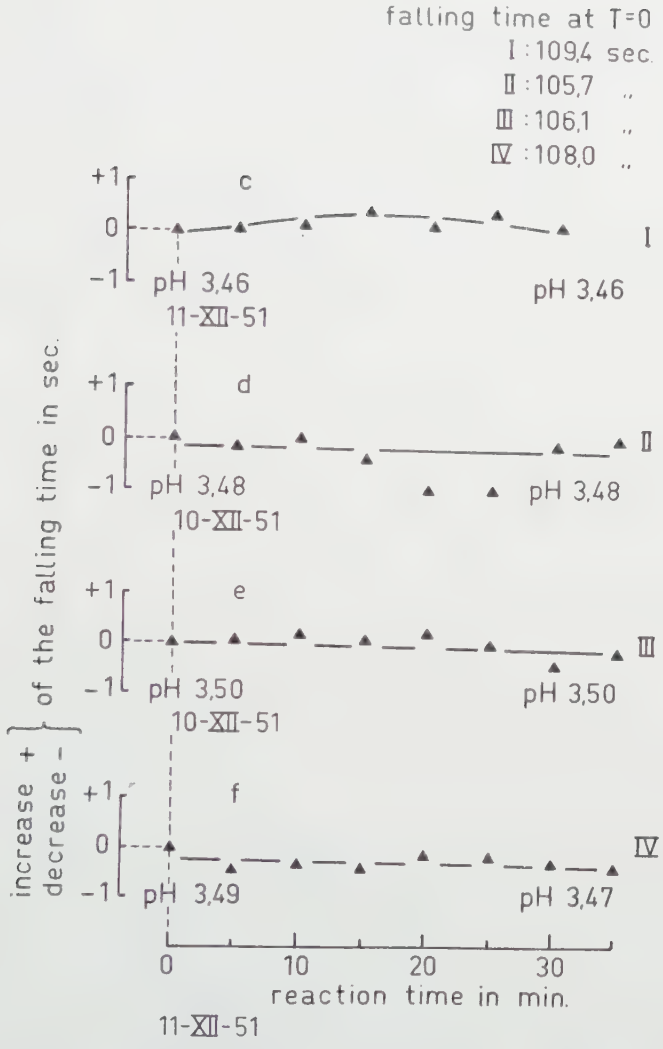

Fig. 6. The effect of different reagents on the viscosity of a pectic acid solution uninfluenced by pectase.

50 ml 0,14 % M.P.Z.-pectic acid in 0,05 mol. succinic acid-borax buffer, pH 3,6. Temp. 25° C.

a. 5 ml inhibitor solution containing 25 mg RI; 1 ml 1 % Pectasin-A.
b. 5 ml inactivated inhibitor solution containing 25 mg RI; 1 ml 1 % Pectasin-A
c. 5 ml inhibitor solution containing 25 mg RI; 1 ml water.
d. 5 ml water; 1 ml 1 % inactivated Pectasin-A.
e. 5 ml inactivated inhibitor solution containing 25 mg RI; 1 ml water.
f. 6 ml water.

(11, 13) but the pH values at the beginning and at the end of the experiments given in the figures, indicate that no explanation for the phenomenon can be derived in this way. The same holds true for viscosity changes which might have arisen from mixing the solutions or from accidental salt additions with the RI solutions as is shown by the graphs c, d, e and f.

One more possibility has to be considered. It could be argued that the commercial pectin preparations used in the experiments do contain substances which, apart from the pectins, show in solution a rather high viscosity of their own. The pectinase on the other hand might contain enzymes which break down these unknown compounds

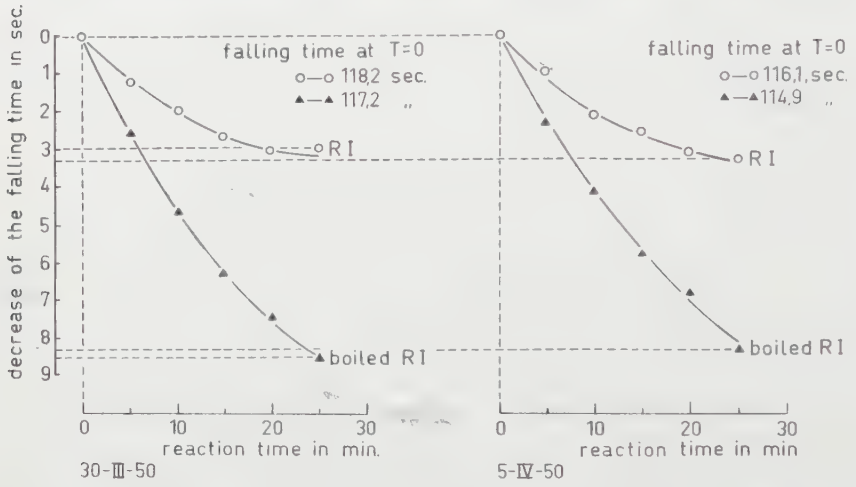

Fig. 7. The effect of the degree of purity of the pectin on the inhibition of pectinase by RI.
50 ml 0,20 % B.P.-pectin (pure pectin 60,9 %) and 0,17 % Z.B.P.-pectin (pure pectin 86,1 %) resp. in 0,05 mol. succinic acid-borax buffer, pH 3,6; 5 ml RI solution containing 1,55 mg RI; 1 ml 1 % Pectasin-A. Temp. 25° C.

and so produce a decrease in viscosity parallel to the one caused by the pectinase. The sap or the RI solution might inhibit these last mentioned enzymes, thus lessening the decrease in viscosity.

An experiment in which the same pectin is used in two different degrees of purity shows that this supposition is not correct since the inhibition of the pectinase is the same whether the purity of the pectin amounts to 61 or 86 % (Fig. 7).

Thus it has been proved beyond doubt that some substance present in the sap of pears is an inhibitor for the enzyme pectinase.

Some properties of the pectinase inhibitor

When increasing amounts of the inhibitor are added to replicate mixtures of pectin and pectinase, the inhibition of the enzyme is found to increase almost linear with the lower concentrations of the inhibitor.

Above a certain amount, however, no further increase in inhibition

is met with (Fig. 8, a and b). If these experiments are repeated with pectic acid as a substrate the same results are obtained. Whereas the maximum inhibition with pectin amounts to 82 %, for pectic acid the inhibition is only 50 %. So the substrate seems to be an important factor in the inhibition.

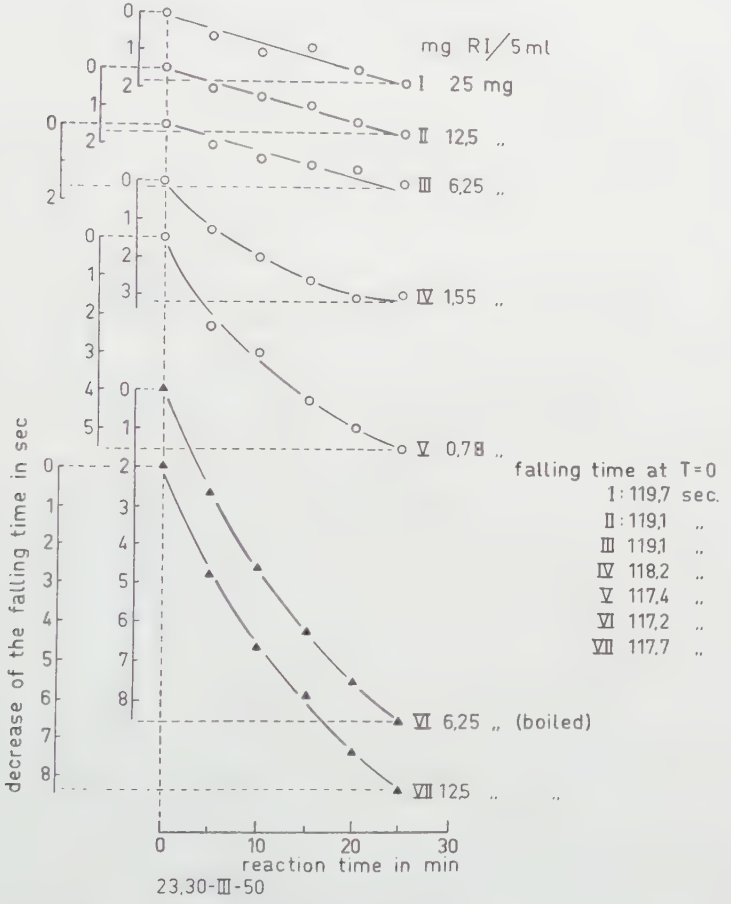

Fig. 8a. The effect of different amounts of inhibitor on the decrease in viscosity of a pectin solution caused by pectinase.
50 ml 0,20 % B.P.-pectin in 0,05 mol. succinic acid-borax buffer, pH 3,6; 5 ml inhibitor solution containing different amounts of RI; 1 ml 1 % Pectasin-A. Temp. 25° C.

This is supported by the results of experiments presented in the Figs. 9, 10 and 11 where pectins from different sources are used. With the pectin prepared from pears, as with the apple pectin used in the experiments described above, a marked inhibition of the enzyme was found. The experiments with pectins from oranges and lemons show a very weak or no inhibition. Data about the properties of the pectins,

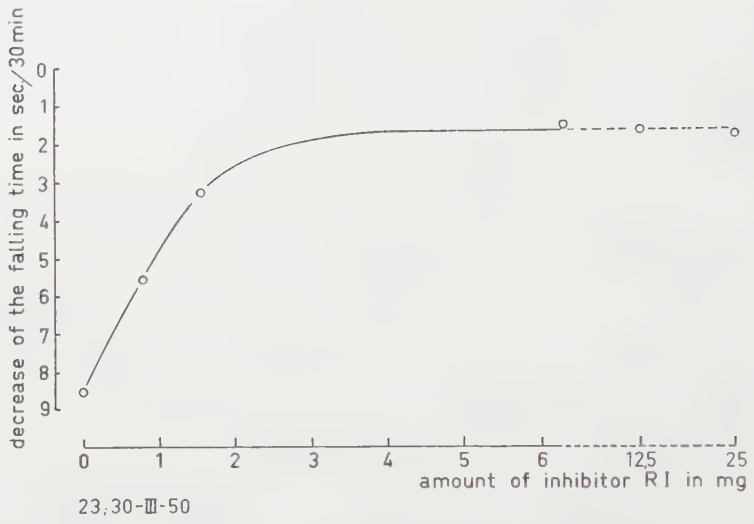

Fig. 8b. The relation between the decrease in viscosity of a pectin solution caused by pectinase and the amount of inhibitor.
Consolidation of the results given in Fig. 8a.

TABLE I

Notation	Type of pectic material	Ash in %	Free carboxyl	Carbox. esterified	Equivalent weight	Degree of esterification in %	pure pection in %
B.P.	Apple pectin commercial	4,3	0,097	0,236	628	70,4	60,9
Z.B.P.	B.P. purified.	0,4	0,147	0,317	586	68,3	86,1
M.P.	Apple pectin commercial	6,6	0,162	0,237	454	59,8	73,5
Z.M.P.	M.P. purified	0,5	0,212	0,261	410	55,5	86,9
M.P.Z.	Pectic acid prep. from M.P.	2,2	0,472	0,005	178	1,2	84,0
C.P.	Citrus pectin commercial	4,3	0,097	0,134	450	58,5	43,5
S.P.	Orange pectin prep. from orange albedo	1,1	0,090	0,316	847	77,9	75,8
P.P.	Pear pectin prep. from pears	0,5	0,072	0,247	830	77,0	59,6

determined according to DEUEL (3) are presented in Table 1; they offer no explanation for the differences in inhibition encountered.

The phenomenon of a maximum inhibition, by which a certain amount of breakdown of the substrate is uninfluenced by further

additions of RI, cannot be explained by the experiments given. It is possible that the commercial pectinase used may be a mixture of several enzymes, all of which lower the viscosity of pectin solutions but only some of which are affected by the inhibitor. Apart from McColloch and Kertesz, other investigators have pointed out the possible existence of more then one pectinase (1, 12, 14, 17). As a matter of fact a preliminary experiment with the pectinase ("depolymerase") prepared from tomatoes according to McColloch and

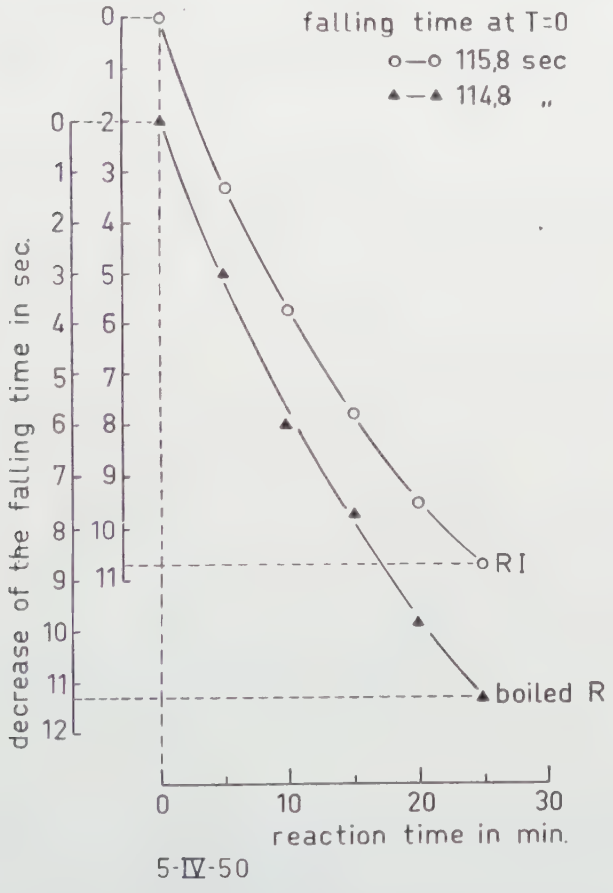

Fig. 9. The effect of the inhibitor on the decrease in viscosity of a citrus-pectin solution caused by pectinase.
50 ml 0,34 % C.P.-pectin in 0,05 mol. succinic acid-borax buffer, pH 3,6; 5 ml inhibitor solution containing 1,55 mg RI; 1 ml 1 % Pectasin-A. Temp. 25° C.

Kertesz showed no inhibition of the enzyme by the RI when pectic acid was used as a substrate (Fig. 12).

By comparing the influence of cystein-activated papainase and the inhibitor on the activity of pectinase, it can be demonstrated that the

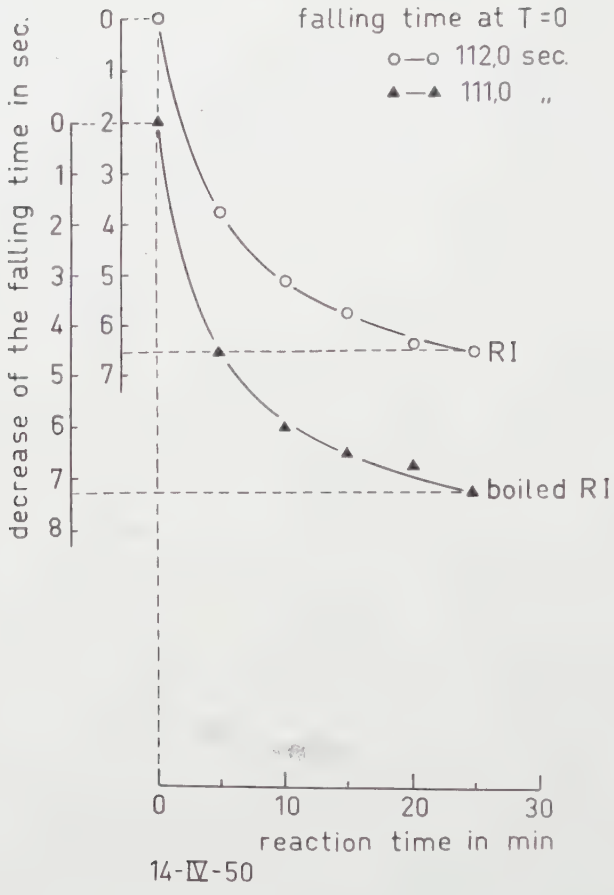

Fig. 10. The effect of the inhibitor on the decrease in viscosity of an orange-pectin solution caused by pectinase.
50 ml 0,08 % S.P.-pectin in 0,05 mol. succinic acid-borax buffer, pH 3,6; 5 ml inhibitor solution containing 1,55 mg RI; 1 ml 1 % Pectasin-A. Temp. 25° C.

inhibition is not caused by an enzymic breakdown of the protein body of the pectinase. The experiments are presented in detail elsewhere (16).

It was also shown that when a solution of malic acid was added to a mixture of pectin and pectinase no change in the activity of the enzyme was found when concentrations of the acid were applied comparable with those of the inhibitor (CHONA (2)).

The sap of two other pear varieties (*Comtesse de Paris*, *Fondante de Charneu*) which were tested in this respect were also found to contain the inhibitor.

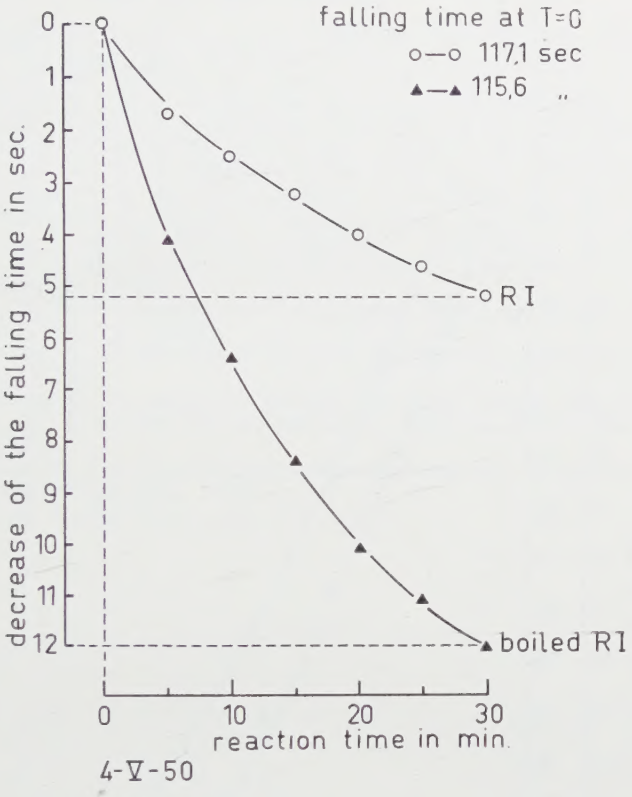

Fig. 11. The effect of the inhibitor on the decrease in viscosity of a pear-pectin solution caused by pectinase.
50 ml 0,06 % P.P.-pectin in 0,05 mol. succinic acid-borax buffer, pH 3,6; 5 m inhibitor solution containing 1,55 mg RI; 1 ml 1 % Pectasin-A. Temp. 25° Cl

Summary

In the sap of several varieties of pears a thermolabile inhibitor for the enzyme pectinase is found. The inhibitor can be isolated by precipitation with acetone. The inhibition of the enzyme increases with increasing amounts of the inhibitor up to a maximum, above which no further increase in inhibition is found. The rate of the residual breakdown of the pectin is influenced by the properties of the substrate.

Evidence from the experiments points to the existence of different kinds of pectinase.

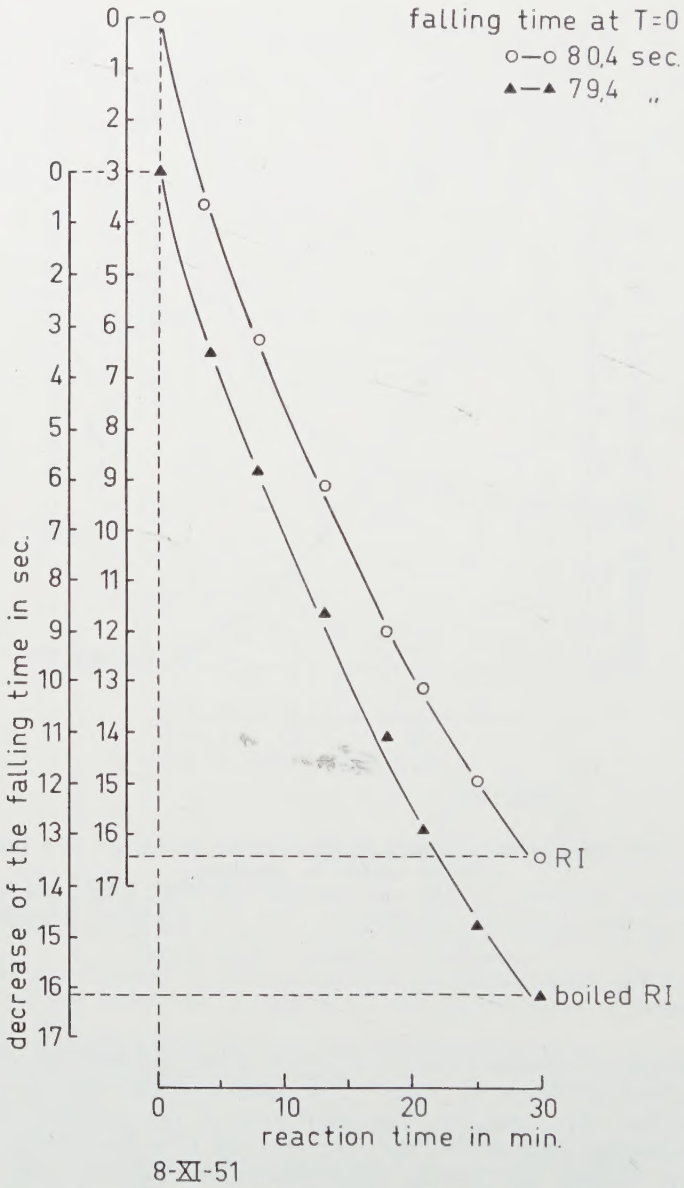

Fig. 12. The effect of the inhibitor on the decrease in viscosity of a pectic-acid solution caused by tomato-pectinase.
50 ml 2 % M.P.Z.-pectic-acid in water, pH 4,0; 5 ml inhibitor solution containing 50 mg RI; 5 ml "depolymerase" extract. Temp. 25° C.

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